AWARD NUMBER: W81XWH-14-1-0251

TITLE: Exploring the Hypersensitivity of PTEN Deleted Prostate Cancer Stem Cells to WEE1 Tyrosine Kinase Inhibitors

PRINCIPAL INVESTIGATOR: Dr. KIRAN MAHAJAN

CONTRACTING ORGANIZATION: H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida 33612

REPORT DATE: December 2015

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2015	Final	30 Sep 2014 - 29 Sep 2015

4. TITLE AND SUBTITLE

Exploring the Hypersensitivity of PTEN Deleted Prostate Cancer Stem Cells to WEE1 Tyrosine Kinase Inhibitors

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0251

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Kiran Mahajan

E-Mail:kiran.mahajan@moffitt.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
H. Lee Moffitt Cancer Center & Research Institute
12902 Magnolia Drive
Tampa Fl 33612

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Central to all cycling cells-including prostate cancer stem cells- is the expression of WEE1 tyrosine kinase. WEE1 monitors duplication of the chromatin during each cell cycle to preserve genome stability and prevent mitotic catastrophe. PTEN (phosphatase and tensin homolog) is deleted at the 10q23.3 locus in ~40% of human prostate cancers and is associated with aggressive metastatic disease with poor prognosis and androgen-independence. A PTEN null prostate cancer derived cell line, LNCaP, displays hypersensitivity and undergoes significant cell death in response to treatment with the WEE1 inhibitor, MK1775. In contrast to LNCaP, MK1775 induces a differentiation like phenotype in the PTEN wildtype prostate cancer derived cell line, LAPC4. Our hypothesis is that PTEN deletion results in hyper-proliferation phenotype in part due to the constitutive activation of the oncogenic AKT survival kinase and these cells require WEE1 to ensure proper chromatin duplication. However, blocking WEE1 function, will force cells to enter mitosis with incompletely replicated chromatin leading to mitotic catastrophe. In contrast, LAPC4 cells, with regulated AKT activation will respond to WEE1 inhibition by undergoing arrest at the G2/M border. This proposal will explore WEE1 kinase as a novel therapeutic target in PTEN mutated prostate cancer. The main objectives are to first examine WEE1 nuclear signaling in isogenic PTEN-deficient and proficient prostate cancer cell lines and prostate cancer stem cell population. Second, interrogate whether PTEN depleted prostate cancer stem cells (PCSC) display enhanced sensitivity to WEE1 inhibitors. Finally, determine whether WEE1 inhibitors prevent tumorigenic potential of PCSC and block metastasis of PTEN null prostate xenograft tumors. The results from these studies will reveal whether targeting PTEN-deficient human tumors with WEE1 inhibitors can induce specific lethality of PCSCs, with limited toxicity to the neighboring PTEN proficient prostate epithelial cells or the stromal cells.

15. SUBJECT TERMS
Prostate Cancer PTEN tumor suppressor WEE1 tyrosine kinase MK1775 Cell cycle

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	41	19b. TELEPHONE NUMBER *(include area code)* 301-619-0523

Table of Contents

Page

1. Introduction……. 1

2. Keywords……. 2

3. Accomplishments……...……... 2

4. Impact……...…… 13

5. Changes/Problems...…..…… 14

6. Products…….……..…..…….14

7. Participants & Other Collaborating Organizations……. 15

8. Special Reporting Requirements…… 15

9. Appendices…… 15

INTRODUCTION:

Prostate Cancer (PC) is a commonly diagnosed cancer with 233,000 new cases each year and a second leading cause of cancer related deaths among American men [1]. While early stage organ confined PCs may be curative with surgery or radiotherapy, advanced prostate cancers are linked to poor prognosis [1, 2]. Advanced disease is treated either with Androgen Deprivation Therapy (ADT)- surgical or chemical castration with anti-androgens and/or radiotherapy [2, 3]. The molecular mechanisms by which PC cells evade treatment appear to be varied with the cells activating alternative survival pathways in response to specific treatment/s and thus present a formidable challenge for PC therapy [2, 4-8]. Cancers are presumed to be driven by cancer stem-like cells (CSC), or tumor-initiating cells, which often constitute <0.1 % fraction of a total cancer cell population [9, 10]. CSCs in many solid tumors, including cancers of the prostate, have been identified using the cell surface adhesion molecule CD44+ve PSAlo, either individually or in combination with other cell surface markers such as CD133, integrin α2β1, and ALDH1A1 [9, 11].. Prostate Cancer Stem Cell-like population (PCSCs) with enhanced self-renewal, clonogenic, tumor-initiating and metastatic capacities are enriched for CD44+ve PSAlo expression [12] [9, 13-16].

Central to all cycling cells-including prostate cancer stem cells- is the expression of WEE1 tyrosine kinase. This evolutionarily conserved tyrosine kinase monitors both DNA and histone synthesis during each cell cycle to ensure proper duplication of chromatin [17]. Interference of these two events results in genome instability and mitotic catastrophe. A potent and highly selective cell cycle and WEE1 selective inhibitor that is currently in Phase I clinical trials in solid tumors is MK1775 (AZD1775).

PTEN (phosphatase and tensin homolog) is deleted at the 10q23.3 locus in ~40% of human prostate cancers and is associated with aggressive metastatic disease with poor prognosis and androgen-independence. Some prostate cancer cell lines, such as LNCAP which harbor PTEN deletion, display hypersensitivity to the WEE1 inhibitor MK1775 and undergo cell death, while the same inhibitor induces differentiation like phenotype *i.e.* permanent arrest of another prostate cancer derived cell line, LAPC4 that harbor wild type PTEN. Our hypothesis is that PTEN deletion results in hyper-proliferation phenotype in part due to constitutive activation of the survival kinase AKT.

Because of this, PTEN null cycling cells may be exquisitely sensitive to WEE1 inhibitors. Thus, prostate cancer patients who harbor PTEN deletions are more likely to respond to WEE1 inhibitors.

Hypothesis: Our hypothesis is that proliferating prostate cancer cells and the pluripotent prostate cancer stem cells require WEE1 to ensure proper chromatin duplication. Targeting these cells with WEE1 inhibitors will induce mitotic catastrophe, with limited toxicity to differentiated cells.

KEYWORDS: Prostate cancer, PTEN tumor suppressor, WEE1 tyrosine kinase, MK1775, Cell cycle

ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. Examine WEE1 and AKT signaling in isogenic PTEN-deficient and proficient prostate cancer cells and prostate cancer derived stem cell population.

Aim 2. Interrogate whether PTEN depleted prostate cancer stem cells display enhanced sensitivity to WEE1 inhibitors.

Aim 3. Investigate the cytotoxicity of WEE1 inhibitors against recalcitrant xenograft tumors formed by PTEN- PSAlo PCSCs in castrated male immunocompromised mice.

What was accomplished under these goals?

Aim 1. Examine WEE1 and AKT signaling in isogenic PTEN-deficient and proficient prostate cancer cells and prostate cancer derived stem cell populations.

a. Generation of isogenic PTEN deficient and proficient human prostate cancer cell lines

A major aspect of this proposal was to generate the isogenic human prostate cancer cell lines. After two unsuccessful attempts with shRNA methodology, we were finally able to generate the PTEN deficient LAPC4 cell lines using the CRISPR/Cas9 based technology as described below. Similarly, we found that LNCaP cells restored with the PTEN grew poorly. However, we were successful in generating a PTEN expressing LNCaP-C42B cell line, a metatstatic derivative of LNCaP.

We generated the isogenic WT and PTEN knockout LAPC4 cell lines using CRISPR/Cas9 gene editing technology. LAPC4 cell lines were co-transfected with the CRISPR/Cas9 and PTEN HDR plasmids. The PTEN HDR plasmid provides a specific DNA repair template. This template contains two 800 base pair homology arms corresponding to distant regions of the PTEN genomic DNA, that are designed to recombine with the genomic DNA after double strand breaks are created by the CRISPR/Cas9 DNA endonuclease. In addition, the plasmid includes the Purmoycin resistance gene for selection and Red fluorescent protein (RFP) to visualize transfection. Following DNA repair and recovery of the cells after Cas9-induced DSBs, puromycin was added to select for positive transfectants (**Figure 1 A**). 8 Stable individual PTEN edited clones referred to as LAPC4 PTENKO clones 1 to 8 were selected after isolation by flow cytometry followed by serial dilution (**Figure 1 B-D)**. Knockdown of PTEN expression in the 8 clones was confirmed by immunoblotting with PTEN specific antibodies **(Figure 1 D)**.

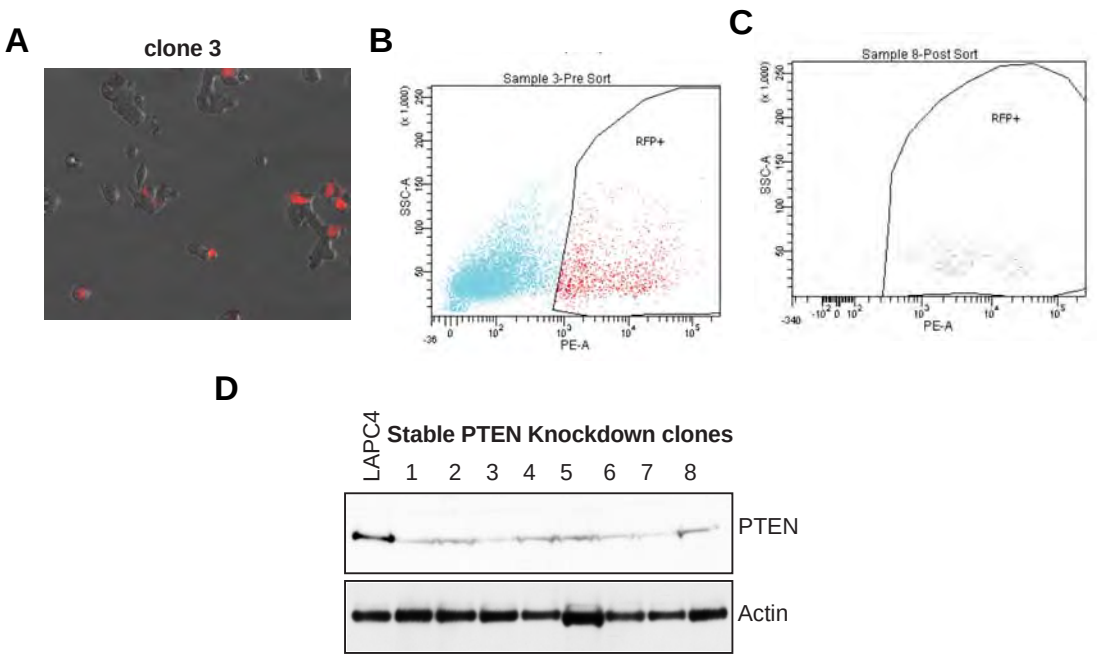


Figure 1: Generation of isogenic PTEN or PTEN KO LAPC4 cell lines. A. Stable PTEN knockdown (PTEN KO) clones are RFP positive as observed by fluorescence microscopy. **B and C**. Individual clones were isolated using flow cytometry based sorting and expanded. **D**. Knockdown of PTEN expression in 8 clones was confirmed by immunoblotting with PTEN specific antibodies. Actin was used as a loading control.

b. Generate isogenic LNCaP cell lines in which PTEN function has been restored

Next, we generated LNCAP and LNCAP PTEN plus and LNCaP-C42B and LNCaP-C42B PTEN cell lines. LNCAP and its metastatic derivative C4-2B harbor PTEN deletion. PTEN is a negative regulator of AKT signaling and thus LNCAP cells display deregulated activation of AKT as seen in the increased serine 473 phosphorylation. LNCaP PTEN proficient clones grew very slowly and formed very small colonies. In contrast, reintroduction of PTEN into the LNCAP C42B cell line which is a metastatic derivative of LNCaP was able to overcome the powerful effect of PTEN functional restoration and cells were able to grow and divide. Reintroduction of PTEN suppressed AKT serine 473 phosphorylation in the C42B background (**Figure 2A**). In contrast, deletion of PTEN leads to hyper-phosphorylation of AKTser473 in the LaPC4 background (**Figure 2B**). Cdc2/Cdk1 is a substrate of WEE1 kinase. Higher levels of Cdc2Y15 phosphorylation was observed in C42B-PTEN expressing cells compared to C42B that is abrogated in both cases following treatment with 5uM of MK1775. Phosphorylation of Cdc2Y15 in the absence of exogenous DNA damage is generally indicative of cells undergoing replication stress response during S phase. As a consequence cells are known to activate WEE1 signaling mediated G2/M checkpoint to allow for repair of replication stress induced DNA damage before entry into mitosis.

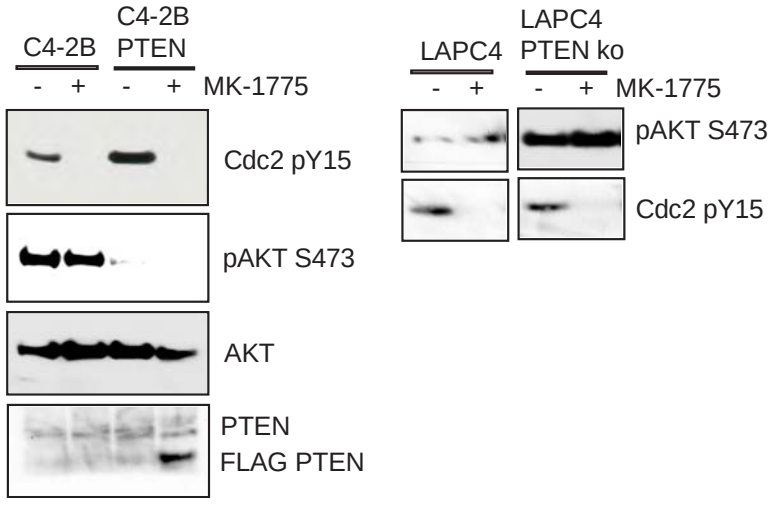


Figure 2: WEE1 and AKT signaling in isogenic PTEN-deficient and proficient prostate cancer cells. A. C42B cell lines stably expressing PTEN and the parental C42B **(B)** The LAPC4 WT and the LAPC4 PTEN deficient cell lines were treated or untreated with the WEE1 selective small molecule inhibitor MK1775 (5uM) for 16 hours. Whole cell extracts were prepared and analyzed by immunoblotting. Cdc2Y15 phosphorylation is indicative of WEE1 activity in these cell lines that is abrogated following treatment with the WEE1 inhibitor MK1775. Reintroduction of PTEN suppressed AKT serine 473 phosphorylation in the C42B background. Deletion of PTEN leads to hyper activation of AKT in the LAPC4 background with no apparent effect on WEE1 signaling.

c. Isolation of CD44+ve PSAlo positive prostate cancer stem-like cells

We enriched CD44+ve PSAlo^{+} population from androgen dependent LNCaP, castration resistant C4-2B and androgen dependent LAPC4 cells by FACS sorting. Representative sorts are shown below (**Figure 3 and table 1**). The CD44+ve PSAlo population in LNCaP and LaPC4 were less than 0.5-1% of total population.These cells expressed very low levels of PSA (Table 1) In contrast, 3-5% of C4-2B cells were CD44+ve PSAlo positive. These CD44+ve PSAlo positive cells showed similar patterns of AKT S473 phosphorylation as bulk population that was dependent on PTEN expression.

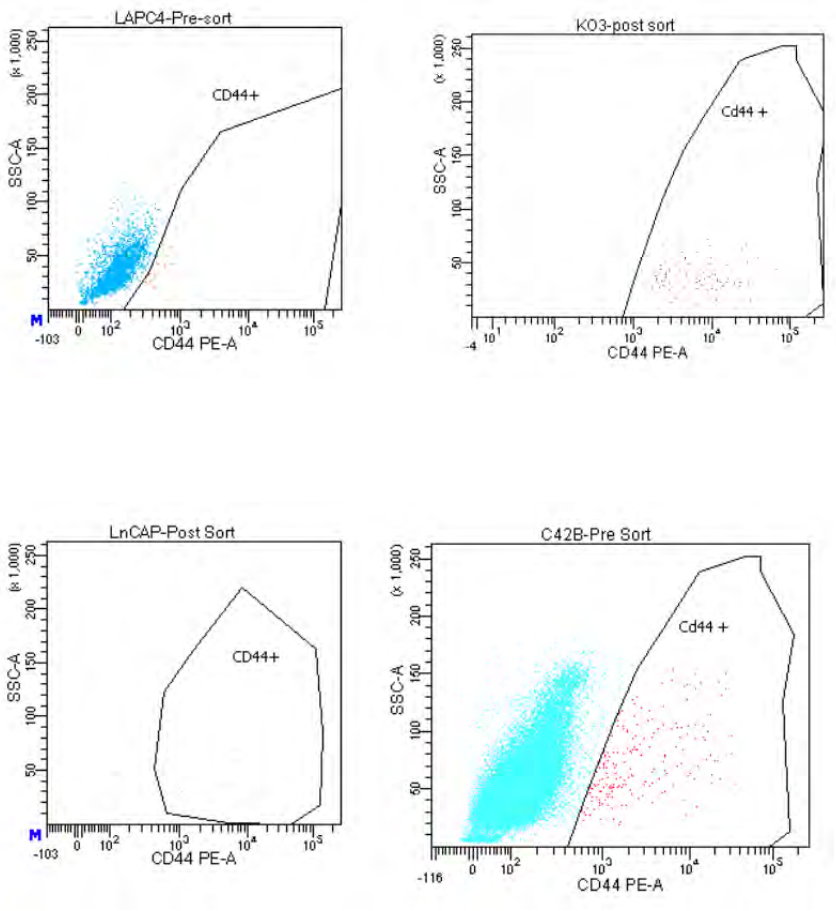


Figure 3. **Isolation of PCSCs**. CD44^{+ve} LAPC4, LAPC4 PTEN KO (KO3), LNCaP and C42B cells were isolated by sorting cells following staining with the CD44-PE antibodies. About 500-5000 cells could be sorted from 5 million cells.

Advanced prostate cancers are treated with chemotherapy and/or radiotherapy. To test the efficacy of WEE1 inhibitors against the refractory PCSC-like cells, we treated CD44+ve PSAlo enriched LNCaP cells with 5uM of inhibitors, MK1775 (WEE1 selective), KU55393 (ATM-selective) or Olapirib (PARP-selective) for 24 hours and irradiated them at 4Gy and cell viability was assessed

(**Figure 4**). CD44+ve PSAlo PCSCs LNCaP cells are exquisitely sensitive to WEE1 inhibitors (5uM) even in the absence of DNA damage. In contrast, these cells showed a refractory population with ATM and PARP inhibitors at the same inhibitor concentrations

Table 1	**PSA/Actin mRNA expression**
LNCaP CD44+ve	3.7×10^{-5}
LNCaP	0.76

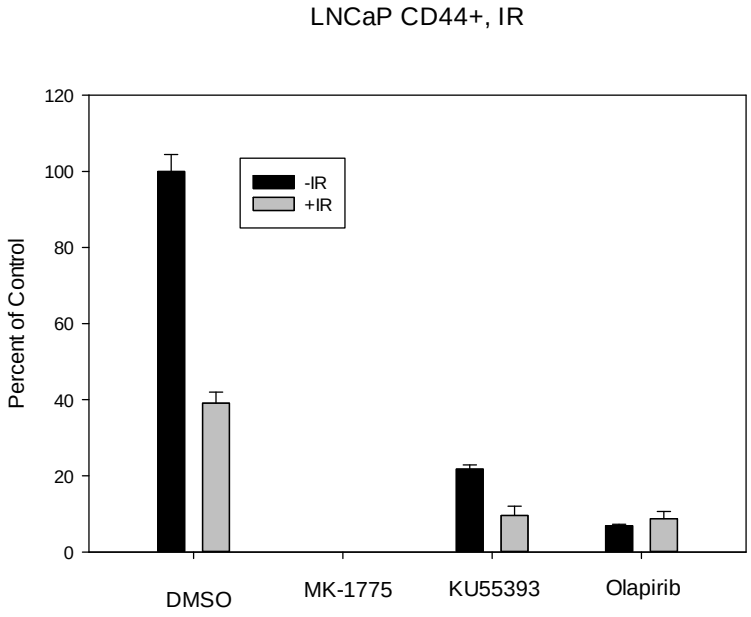


Figure 4: CD44PCSCs display exquisite sensitivity to WEE1 inhibitors. LNCaP derived $CD44^{+ve}$ PCSCs were treated with various inhibitors in the presence and absence of IR (4GY) and cell viability was determined by Trypan blue assay.

Western blot analysis revealed regulated activation of AKT in CD44+ve PSAlo PTEN positive cells C42B cells compared to uncontrolled activation in the isogenic C42B background.

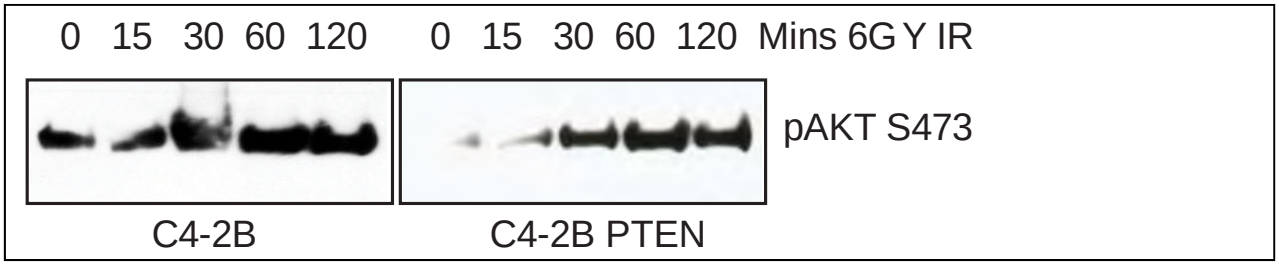
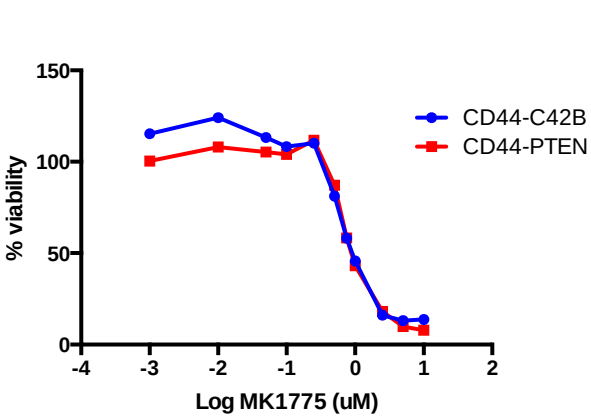


Figure 5: AKT activation is tightly regulated upon PTEN restoration. CD44 positive C42B or C42B-PTEN were treated with 5 GY IR and harvested at various time points post-irradiation. AKT is constitutively phosphorylated at serine 473 in C42B cells and increases marginally upon DNA damage. In contrast, a time dependent increase in AKT phosphorylation was observed in C42B-PTEN PC cell line.

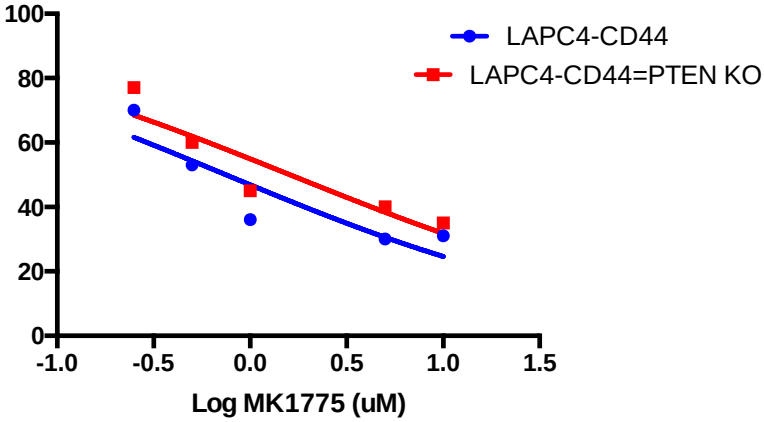
Aim 2. Interrogate whether PTEN depleted prostate cancer stem cells display enhanced sensitivity to WEE1 inhibitors

a. Viability of isogenic pairs of PCSC cells upon treatment with the WEE1 inhibitor MK1775

Isogenic pairs of PTEN proficient and deficient PCSCs and bulk C4-2B populations were treated with MK1775 at various concentrations for 96 hrs and cell viability was determined using cell titre Glo assay.



	CD44-C42B	CD44-PTEN
IC50	0.9695	0.9481



	IC50
LAPC4-CD44	0.7484
LAPC4-CD44=PTEN KO	1.606

Figure 6: Dose response curves of CD44 positive PTEN WT and deficient PCSCs to WEE1 inhibitors. Isogenic pairs of PTEN proficient and deficient CD44 PCSCs and bulk C42B populations or LAPC4 parental and PTEN KO were treated with MK1775 for 96 hrs and cell viability was determined using cell titre glo assay (*n* = 8 replicates).

While the CD44+ve PSAlo positive cells C4-2B or C4-2B-PTEN displayed similar half maximal inhibitory concentration IC_{50s} to the WEE1 inhibitor at higher concentrations, at lower concentrations the CD44+ve PSAlo C4-2B showed resistance to MK1775 compared to CD44+ve PSAlo-PTEN proficient cells. In contrast, the difference is more profound in LAPC4 derived PCSCs which showed that LAPC4PTEN KO CD44+ve PSAlo were ~2 fold more resistant to MK1775 compared to parental LAPC4 CD44+ve PSAlo. Thus hyper activation of AKT is conferring a survival benefit to these cells. Whether this resistance is due to differential activation of checkpoint pathways in these two different genetic backgrounds of PC cells, remains to be addressed.

b. Assess the effect of WEE1 inhibition under condition of radiation induced DNA damage upon PTEN restoration in prostate cancer

To further understand the contribution of WEE1 to prostate cancer cell survival, paired PTEN proficient and deficient C42B were treated with MK1775 and irradiated. Loss of PTEN tumor suppressor protected C4-2B from irradiation induced toxicity. In contrast, the PTEN proficient C4-2B were hypersensitized to WEE1 inhibitor following radiation induced DNA damage (**Table 1**).

.

Table 2: Isogenic PC cell lines	-IR (IC_{50}) + MK1775	+IR (5GY) (IC_{50}) +MK1775
LNCaP-C42B	1 uM	>5uM
LNCaP-C42B+PTEN	1.5 uM	0.6uM

Table 2: Comparison of sensitivity of isogenic PTEN proficient and deficient C4-2B cell lines to single (MK1775) and combination (MK1775+5GYIR) treatments.

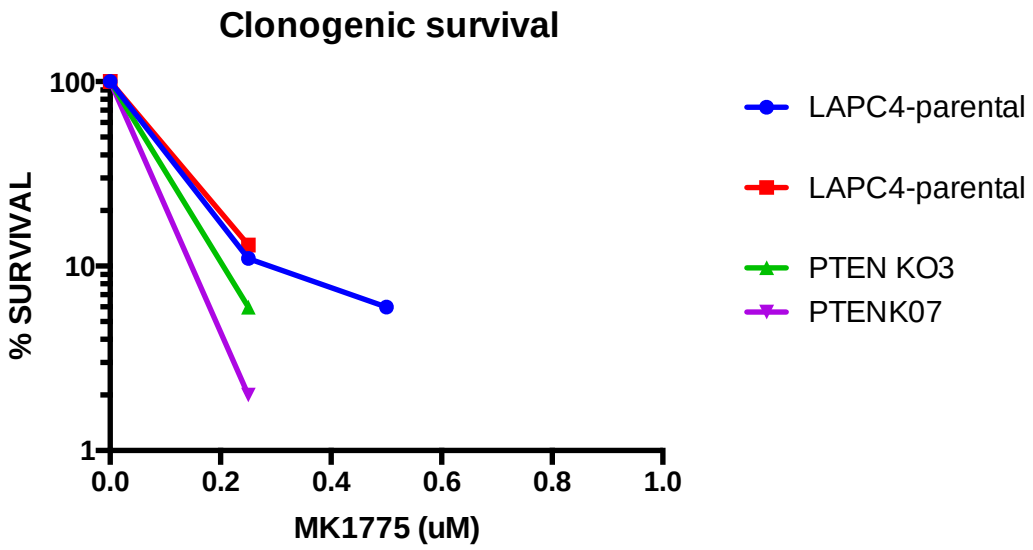


Figure 7: Clonogenic survival assay of PTEN proficient and deficient LAPC4 (PTEN KO3 and KO7) cell lines. Isogenic PTEN proficient (LAPC4 parental) and PTEN deficient LAPC4 were treated with various concentrations of MK1775 and plated. Individual colonies were counted after staining cells with crystal violet. An average of 3 counts is plotted for each concentration and each cell line.

We performed the clonogenic survival assay using LAPC4 as a model as these cell form well defined colonies compared to C42B cells. This assay revealed that PTEN deficient LAPC4 colonies were more sensitive to the cell cycle and epigenetic WEE1 inhibitor MK1775 compared to the isogenic proficient cells (**Figure 7**). Thus LAPC4 cells that have activation of AKT due to PTEN deletion appear to be more susceptible to the WEE1 inhibitors consistent with the sensitivity seen in C4-2B compared to C42B-PTEN cells that had been treated with MK1775 alone (**Table 1**). Inhibition of WEE1 may induce a mitotic catastrophe in the PTEN deficient background.

c. Evaluate PTEN dependency on cell cycle progression following IR and WEE1 inhibitor treatment in metastatic PCs

To understand the reason for this differential radio sensitivity between PTEN proficient and deficient PC cells in response to treatment with WEE1 inhibitor, we analyzed the cell cycle profiles (**Figure 8**). Following MK1775 treatment, the C42B cells were predominantly arrested in S phase (orange bars). However upon IR treatment (5Gy), the number of cells in S phase decreased and the number of cells in G2 were increased (green bars). In cells that were treated with both MK1775 and IR the number of cells undergoing G2/M decreased and the number in S phase increased. Interestingly, in C42B-PTEN positive cells, regardless of treatment all the cells were found to be stuck in G1 (brown bars). These results suggest that in the absence of G2/M checkpoint, DSBs induced by radiation in a PTEN deficient background stall cells in S phase that may eventually activate alternative DNA repair pathways and the emergence of radioresistant populations.

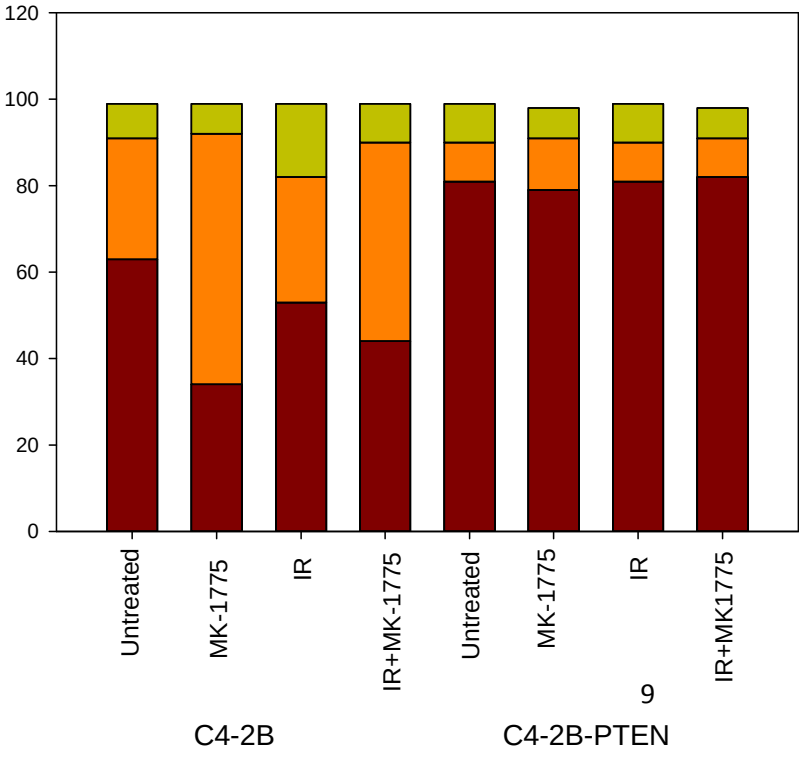


Figure 8: Cell cycle analysis of isogenic C4-2B and C42B-PTEN treated with WEE1 inhibitors. C42B or C42B-PTEN were treated with 5 GY IR and harvested at 2 h post-irradiation.

d. Screen for clonogenic potential and sphere-forming ability in the presence of WEE1 inhibitors

i. We next examined the sensitivity of isogenic pairs of PTEN deficient PCSCs using clonogenic survival assays, which measures the reproductive ability of individual cells to reproduce indefinitely and form colonies While LAPC4 CD44+ve PSAlo positive cells formed clearly outlined colonies from single cells on non-adherent surface the C42B CD44+ve PSAlo were diffuse (**Figure 9**).

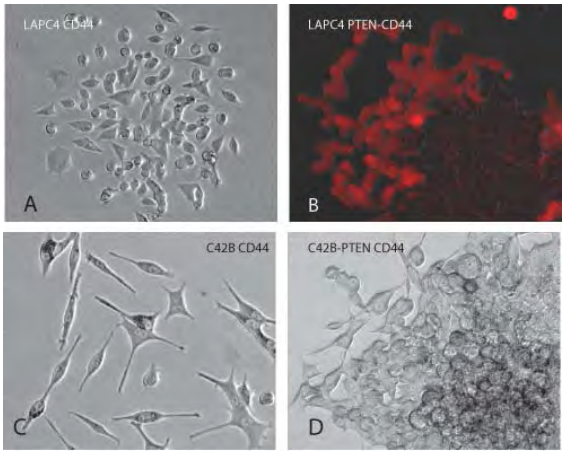


Figure 9: Morphological properties of PTEN deficient and proficient CD44 PCSC colonies on adherent surfaces. Single cell colonies of CD44 positive LAPC4 or LAPC4PTENKO3 or C42B or C42B+PTEN were allowed for two weeks to establish and visualized by microscopy. Addition or deletion of PTEN significantly changed the morphology of this population. C42B CD44 positive cells that were more dispersed tended to coalesce. Interestingly, some of the LAPC4 PTEN KO cells showed heterogeneity for RFP fluorescence.

ii. Isogenic pairs of flow cytometry purified CD44+ve PSAlo positive PTEN proficient and deficient PC cells were plated on adherent and non-adherent surfaces. Sphere formation assay is used to evaluate the self renewal and differentiation at a single cell level on non adherent surfaces. In the control, DMSO treated populations, the CD44+ve PSAlo positive LNCaP cells formed robust spheres. Upon treatment

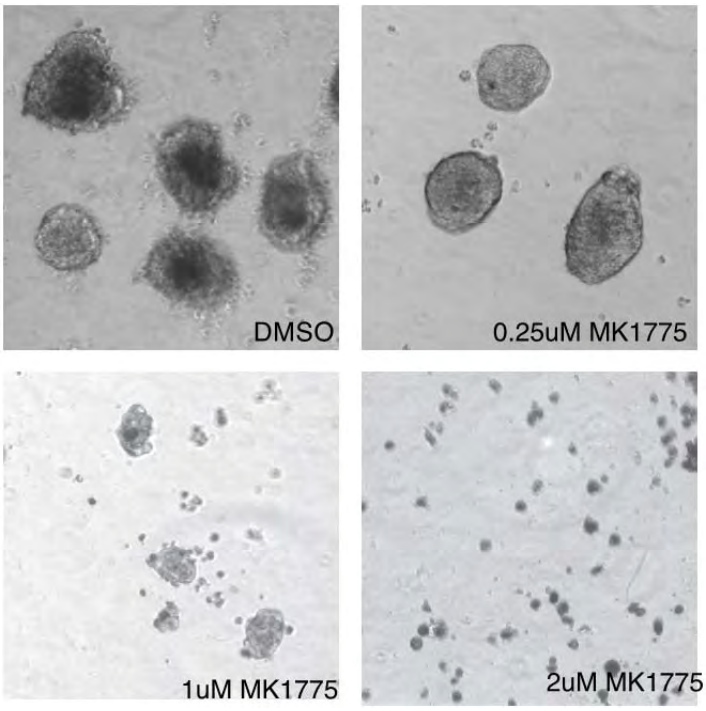


Figure 10: LNCaP CD44 PCSC treated with MK1775. CD44 positive LNCaP were treated with control (DMSO).or increasing concentrations of MK1775. The sphere formation was completely abrogated at 2uM of MK1775.

with increasing concentrations of MK1775 the sphere forming ability was gradually diminished. At 1uM concentration, the spheres appeared fragmented and at 2 uM spheres were completely abolished (**Figure 10**). In contrast to the CD44+ve PSAlo population derived from the non-metastatic LNCaP, the CD44+ve PSAlo population from the metastatic C42B and CD44+ve PSAlo C42B-PTEN were exquisitely sensitive to MK1775 at 0.25 uM concentration. Thus WEE1 activity is integral for stem like property of CD44+ve PSAlo positive prostate cancer stem cells.

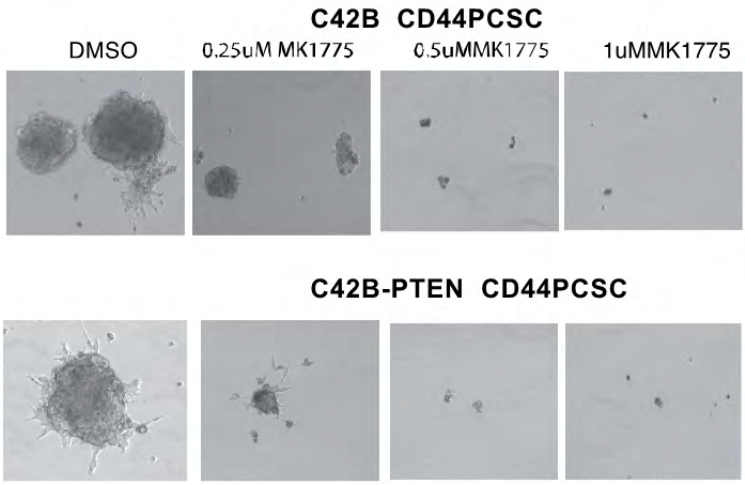


Figure 11: PTEN restoration does not confer protective property against loss of WEE1 function in sphere assay. CD44 positive C42B or C42B-PTEN were plated on matrigel in a 6 well plate and treated with increasing concentrations of WEE1 inhibitor. In control cells robust sphere formation was seen in both genetic backgrounds. However, MK1775was sufficient in blocking sphere formation of CSCs like cells.

Aim 3. Investigate the cytotoxicity of WEE1 inhibitors against recalcitrant xenograft tumors formed by PTEN- CSCs in castrated male immunocompromised mice.

We first analyzed growth of PTEN null xenografts over the control PTEN re-constituted xenografts in castrated and normal male mice. CD44+ve PSAlo positive LNCaP cells (vector, parental or PTEN plus) were injected in normal and castrated male immunocompromised mice (n=6 in each group). Tumor formation was monitored. CD44+ve PSAlo vector and parental LNCaP cells formed xenograft tumors in 50% of the normal male mice by 4 weeks

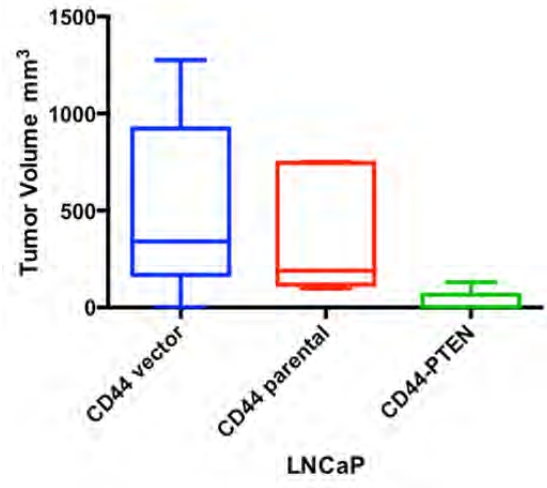


Figure 12: PCSC xenograft growth is inhibited after restoration of PTEN. PTEN deficient LNCaP CD44 cells formed robust tumors in normal mice. Tumor growth was significantly regressed followed reconstitution with the tumor suppressor PTEN.

(Figure 12). Only two of six CD44+ve PSAlo PTEN injected cells formed small tumors (~100mm^3) in castrated male mice after 6 weeks and none formed in other groups. The reason for lack of tumor formation by parental and vector expressing CD44+ve PSAlo LNCaP cells in castrated mice is not known at this time. Mean tumor volume is shown for tumors obtained from 5 of 6 normal mice in each group (**Figure 12**).

b. Immunohistochemical staining of resected tumors for necropsy of the tumor tissue.

Ki67 staining, an indicator of cell proliferation, was performed on control and MK1775 treated tumor sections. While the control tumors were 70-80% Ki-67 positive, MK1775 treated tumors showed small nucleated cells which may represent a sinus histiocytosis, a lymph node reactive response, and no tumor cells. Future studies with other PTEN deficient models are important to validate the effectiveness of WEE1 inhibitors to tackle recurrent PC.

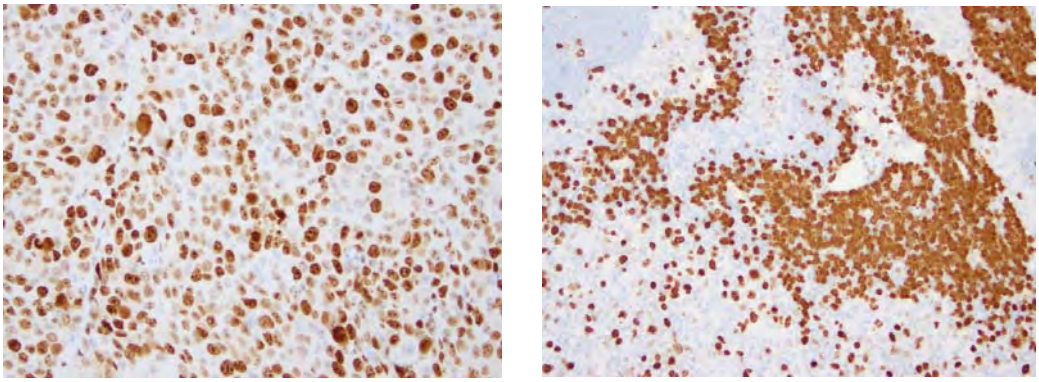


Figure 13: Ki67 staining of control and MK1775 treated xenografts. Immunostaining for Ki67 a cell proliferation marker revealed proliferating cells in control tumors. The MK1775 treated tumors showed very few prostate cancer cells. Majority appeared to be small nucleated cells which may be infiltrating lymphocytes.

CONCLUSIONS

Overall, our results indicate that CD44+ve PSAlo LNCaP prostate cancer stem-like cells (PCSCs) and CD44+ve PSAlo C4-2B PCSCs (a metastatic derivative of LNCaP) are exquisitely sensitive to the WEE1 inhibitor MK1775 (5uM) even in the absence of DNA damage. MK1775, the WEE1 inhibitor currently undergoing Phase II clinical trials for other cancers may be useful in targeting

PTEN deficient prostate cancers and also eliminate the recalcitrant stem cell-like population to overcome resistance to treatment.

1. **What opportunities for training and professional development has the project provided?**
    - *Nothing to Report*
2. **How were the results disseminated to communities of interest?**
    - ***Seminars***
    - Moffitt Cancer Center, Tumor Biology Department Retreat, Tampa, Florida Dec 4, 2015: ***Recalcitrant cancer, cancer stem cells and targeted therapies***.
    - Moffitt Cancer Center, Tumor Biology Research In Progress, Tampa, Florida Nov 3, 2015 : ***Exiting the Comfort Zone: Tackling the Complexity of Castration Resistant Prostate Cancer.***
    -
    - **What do you plan to do during the next reporting period to accomplish the goals?**

"Nothing to Report..

IMPACT:

What was the impact on the development of the principal discipline(s) of the project? This proposal explored WEE1 kinase as a novel therapeutic target in PTEN mutated prostate cancer. Our results indicate that prostate cancer stem-like cells (PCSCs) are exquisitely sensitive to the WEE1 inhibitor MK1775 (5uM) even in the absence of DNA damage. *De novo* loss of a tumor suppressor PTEN protected C4-2B from irradiation induced toxicity. conferred sensitivity in clonogenic survival assays. Loss of PTEN tumor suppressor. In contrast, the PTEN proficient C4-2B were hypersensitized to WEE1 inhibitors following radiation induced DNA damage. PTEN restoration is sufficient to reverse the tumor forming ability of CD44+ve PSAlo positive LNCaP PCSC cells. Thus, WEE1 inhibitors such as MK1775 (AZD1775) currently undergoing Phase II clinical trials for other cancers may be useful in targeting PTEN deficient prostate cancers and also eliminate the recalcitrant stem cell-like populations to overcome resistance to treatment.

What was the impact on other disciplines?

- *Nothing to Report.*

What was the impact on technology transfer?

- *Nothing to Report.*

What was the impact on society beyond science and technology?

- *Nothing to Report.*
    -

CHANGES/PROBLEMS: *Nothing to Report*

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents** *Nothing to Report*
- **Significant changes in use or care of human subjects** *Nothing to Report*
- **Significant changes in use or care of vertebrate animals.** *Nothing to Report*
- **Significant changes in use of biohazards and/or select agents** *Nothing to Report*
-

PRODUCTS: Publications, conference papers, and presentations
Two review articles written during this funding period. **The DOD award was acknowldged in this article.**

Mahajan, K*. and N.P. Mahajan, *Cross talk of tyrosine kinases with the DNA damage signaling pathways.* **Nucleic Acids Res**, 2015.

Mahajan, K.*, *hPso4/hPrp19: a critical component of DNA repair and DNA damage checkpoint complexes.* **Oncogene**, 2015

*Corresponding author

Seminar/Presentations:

1. Moffitt Cancer Center, Tumor Biology Department Retreat, Tampa, Florida Dec 4, 2015: ***Recalcitrant cancer, cancer stem cells and targeted therapies***.

2. Moffitt Cancer Center, Tumor Biology Research In Progress, Tampa, Florida Nov 3, 2015 : ***Exiting the Comfort Zone: Tackling the Complexity of Castration Resistant Prostate Cancer.***

3. Moffitt Cancer Center, Drug Discovery Group Seminar Series, Tampa, Florida, June 23, 2015: ***Epigenetic Underpinnings of the DNA damage response pathways***.

- **Website(s) or other Internet site(s)**. *Nothing to Report*
- **Technologies or techniques** *Nothing to Report.*
- **Inventions, patent applications, and/or licenses** *Nothing to Report*

- **Other Products**
  Generated isogenic pairs of C42B and C42B PTEN plus and LAPC4 and LAPC4 PTEN KO (CRSIPR mediated deletion). These prostate cancer cell lines will be useful in future to investigate various functions of PTEN in prostate cancer survival and metastasis.
-

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Kiran Mahajan*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	KIRAN_MAHAJAN
Nearest person month worked:	*12 months*
Contribution to Project:	*Dr. Mahajan has performed all the experiments in the proposed project.*
Funding Support:	DOD award

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?** No change
- **What other organizations were involved as partners?**
  - *"Nothing to Report."*

SPECIAL REPORTING REQUIREMENTS

NOT APPLICABLE

APPENDICES

REFERENCES

1. Siegel, R., et al., *Cancer statistics, 2014.* CA Cancer J Clin, 2014. **64**(1): p. 9-29.
2. Bahl, A., *Metastatic castration-resistant prostate cancer. Part 1: the challenges of the disease and its treatment.* Eur J Oncol Nurs, 2013. **17 Suppl 1**: p. S1-6.
3. Vlaeminck-Guillem, V., G. Gillet, and R. Rimokh, *SRC: marker or actor in prostate cancer aggressiveness.* Front Oncol, 2014. **4**: p. 222.
4. Antonarakis, E.S., et al., *AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer.* N Engl J Med, 2014. **371**(11): p. 1028-38.
5. Arora, V.K., et al., *Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade.* Cell, 2013. **155**(6): p. 1309-22.
6. Attar, R.M., C.H. Takimoto, and M.M. Gottardis, *Castration-resistant prostate cancer: locking up the molecular escape routes.* Clin Cancer Res, 2009. **15**(10): p. 3251-5.
7. Monti, S., et al., *The IGF axis in prostate cancer.* Curr Pharm Des, 2007. **13**(7): p. 719-27.
8. Mahajan, K. and N.P. Mahajan, *ACK1/TNK2 tyrosine kinase:An emerging target for cancer therapeutics.* AACR Education Book, 2014. **2014**(1): p. 6.

9. Qin, J., et al., *The PSA(-/lo) prostate cancer cell population harbors self-renewing long-term tumor-propagating cells that resist castration.* Cell Stem Cell, 2012. **10**(5): p. 556-69.
10. Lang, S.H., F.M. Frame, and A.T. Collins, *Prostate cancer stem cells.* J Pathol, 2009. **217**(2): p. 299-306.
11. Liu, X., et al., *Systematic dissection of phenotypic, functional, and tumorigenic heterogeneity of human prostate cancer cells.* Oncotarget, 2015.
12. Wicha, M.S., *PSA Lo and Behold: Prostate Cancer Stem Cells.* Cell Stem Cell, 2012. **10**(5): p. 482-3.
13. Patrawala, L., et al., *Highly purified CD44+ve PSAlo+ prostate cancer cells from xenograft human tumors are enriched in tumorigenic and metastatic progenitor cells.* Oncogene, 2006. **25**(12): p. 1696-708.
14. Jeter, C.R., et al., *Functional evidence that the self-renewal gene NANOG regulates human tumor development.* Stem Cells, 2009. **27**(5): p. 993-1005.
15. Collins, A.T., et al., *Prospective identification of tumorigenic prostate cancer stem cells.* Cancer Res, 2005. **65**(23): p. 10946-51.
16. Al-Hajj, M., et al., *Prospective identification of tumorigenic breast cancer cells.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 3983-8.
17. Mahajan, K. and N.P. Mahajan, *WEE1 tyrosine kinase, a novel epigenetic modifier.* Trends Genet, 2013. **29**(7): p. 394-402.

Nucleic Acids Research, 2015 **1**
doi: 10.1093/nar/gkv1166

SURVEY AND SUMMARY

Cross talk of tyrosine kinases with the DNA damage signaling pathways

Kiran Mahajan[1,3,*] and Nupam P. Mahajan[2,3,*]

[1]Tumor Biology Department, University of South Florida, 12902 Magnolia Drive, Tampa, FL 33612, USA, [2]Drug Discovery Department, Moffitt Cancer Center, University of South Florida, 12902 Magnolia Drive, Tampa, FL 33612, USA and [3]Department of Oncological Sciences, University of South Florida, 12902 Magnolia Drive, Tampa, FL 33612, USA

Received June 30, 2015; Revised October 20, 2015; Accepted October 21, 2015

ABSTRACT

Tyrosine kinases respond to extracellular and intracellular cues by activating specific cellular signaling cascades to regulate cell cycle, growth, proliferation, differentiation and survival. Likewise, DNA damage response proteins (DDR) activated by DNA lesions or chromatin alterations recruit the DNA repair and cell cycle checkpoint machinery to restore genome integrity and cellular homeostasis. Several new examples have been uncovered in recent studies which reveal novel epigenetic and non-epigenetic mechanisms by which tyrosine kinases interact with DDR proteins to dictate cell fate, i.e. survival or apoptosis, following DNA damage. These studies reveal the ability of tyrosine kinases to directly regulate the activity of DNA repair and cell cycle check point proteins by tyrosine phosphorylation. In addition, tyrosine kinases epigenetically regulate DNA damage signaling pathways by modifying the core histones as well as chromatin modifiers at critical tyrosine residues. Thus, deregulated tyrosine kinase driven epigenomic alterations have profound implications in cancer, aging and genetic disorders. Consequently, targeting oncogenic tyrosine kinase induced epigenetic alterations has gained significant traction in overcoming cancer cell resistance to various therapies. This review discusses mechanisms by which tyrosine kinases interact with DDR pathways to regulate processes critical for maintaining genome integrity as well as clinical strategies for targeted cancer therapies.

INTRODUCTION

Tyrosine kinase (TK) signaling has garnered a lot of interest in recent years, principally in cancer research, due to the demonstrable success in developing precision drugs to target critical pathogenic drivers (1–4). Under regulated conditions, tyrosine phosphorylation acts as a rapid on-off switch in cells and is employed by the cellular signaling pathways to regulate growth, migration, adhesion, differentiation and survival. Conversely, constitutively active tyrosine kinase signaling cascades relay unrelenting growth and proliferation signals to promote tumor development, progression and metastasis in less than optimal environments. Tyrosine kinases are also known to be activated in cells upon DNA damage and in turn activate signal transduction networks required to restore cellular homeostasis (5–10). These networks comprise of proteins critical for DNA repair, cell cycle checkpoints, chromatin remodeling and restoration, miRNA processing, mRNA splicing and stability (Table 1). Understanding the mechanisms by which tyrosine kinases regulate DDR to impact cell fate in normal cells is essential to delineate their roles in cancer cell resistance to various DNA damaging agents.

DNA double-strand breaks (DSBs) pose a grim threat to cells as loss or gain of genetic material, mutations and chromosome rearrangements due to improper repair can be a primer for malignant transformation (11–14) (Figure 1). In normal cells, DSBs are repaired with high fidelity by members of the homologous recombination (HR) pathway which restore the genetic integrity using a donor template (15). In contrast, when the DSBs are acted upon by members of the non-homologous end joining (NHEJ) pathway, the ends are joined directly with little or no homology and the process is thus error prone. The classical NHEJ or the canonical NHEJ (c-NHEJ) does not rely on the availability

*To whom correspondence should be addressed. Email: kiran.mahajan@moffitt.org
Correspondence may also be addressed to Nupam P Mahajan. Email: nupam.mahajan@moffitt.org

Table 1. Tyrosine kinases–DDR interactions: phosphorylation sites and functional roles

Tyrosine kinase	DDR protein–phosphorylation site	DDR function	References
c-Abl	KAT5 at Y44	Binding to H3K9me3 residue and Activation of ATM by acetylation	(10)
c-Abl	Mdm2 at Y394	Disrupts the binding of MdmX to p53	(150,151)
	HDM2 at Y276	Regulates interaction with ARF	
c-Abl	MdmX at Y99	Disrupts the binding of MdmX to p53	(152)
c-Abl	p73 at Y99, p63 at Y149	Increase in apoptosis and cell death	(69,153,154)
c-Abl	MSH5	Interaction activates Abl kinase and tyrosine phosphorylation of MSH5	(155)
c-Abl	DNA PKcs (pYC-Terminal Domain)	Dissociates the DNA PKcs-Ku complex	(156)
c-Abl	BRCA1 (pYC-Terminal Domain)	BRCA1 negatively regulates Abl tyrosine kinase activity	(157)
c-Abl	Rad51 at Y315	Chromatin association during HRR	(108,109)
c-Abl	Rad52 at Y104	Ionizing radiation induced foci (IRIF) formation	(110)
c-Abl	YAP at the Y357	Regulates p53 dependent apoptosis	(80)
c-Abl	ATM and DNA PKcs	IR induced response	(65,100,104)
c-Abl	HIPK2 at Y360	Regulates p53 dependent apoptosis	(6)
ACK1/TNK2	AR at Y267	Upregulates AR mediated ATM expression	(56,72)
ACK1/TNK2	KDM3A at Y1114	Regulates HOXA1 gene expression	(142)
EGFR	H4 at Y72	Chromatin modulation	(57)
EGFR	ATM at Y370	DNA synthesis and repair, IR induced foci formation	(158)
EGFR	DNAPKcs	NHEJ/radioresistance	(39)
EGFR	PCNA at Y211	Mismatch repair	(61)
IGF-1R	IRS-1	Disrupts IRS-1-Rad51 complex. Required for nuclear translocation of Rad51 during HRR	(30)
Lyn	CDK1, DNA-PK and PKCδ	IR induced activation and association of Lyn - with CDK1. Lyn activates DNA-PK and PKCδ	(63,75–78)
Rad53	H3 at Y99	Regulate histone levels	(87,88)
Src, Fyn, and C-Abl	AKAP8	Chromatin alteration	(121)
Src	ATR/Chk1	Termination of DNA damage response	(31)
WSTF	H2Ax at Y142	Radiosensitization	(112)
WEE1	CDK1 at Y15	G2/M arrest	(159,160)
WEE1	H2B at Y37	Global histone synthesis	(92)

The tyrosine residues in DDR proteins specifically modified by the individual tyrosine kinases are shown in the table. The functional outcome of the tyrosine phosphorylation on the activity of the DDR protein or the corresponding DNA repair pathway is detailed above. Very little cross substrate phosphorylation is observed between individual tyrosine kinases. Each DDR protein appears to be uniquely targeted by a specific tyrosine kinase at distinct tyrosine residues.

of homologous ends for joining DSBs and is catalyzed by Ku complex in conjunction with seven other core members of NHEJ (16–18). Albeit to a lesser degree, some homology-independent repair occurs in the absence of these Ku proteins, and is referred to as the microhomology-mediated end joining (MMEJ), or alternative NHEJ (alt-NHEJ) (19). Even though DNA damage is the initiator of the HR, cNHEJ, and Alt-NHEJ pathways, the activity of the DDR proteins operating in these pathways may be further regulated by post-translational modifications by tyrosine kinases (Table 1). Conversely, inhibition of tyrosine kinase signaling significantly enhances the induction of radiation-induced apoptosis, prevents radiation-induced invasiveness and reverses the radioresistance of tumor cells by interfering with DNA repair (20,21).

Oncogene induced replication stress impairs replication fork progression and therefore activates the DNA damage checkpoints, which function as an anti-cancer barrier (8,22,23). These checkpoints are critical to stall cell cycle progression to allow time for repair, so that cells do not linger with damaged DNA that potentiates genome instability phenotypes (11,24). Cells may either enter replicative senescence or undergo mitotic arrest in the presence of broken chromosomes and shortened telomeres (25–27). However, cancer cells circumvent this anti-tumor barrier by overexpressing positive regulators or mutating the negative regulators of tumorigenesis (28). Tyrosine kinases are also activated in cells following exposure to radiation, interact with DNA repair and checkpoint pathways, to promote survival or apoptosis (29,30). In addition, tyrosine kinases may directly terminate activated checkpoints (31,32). Further, both receptor and non-receptor tyrosine kinases epigenetically regulate DNA damage signaling by modifying the core histones as well as chromatin modifiers (Figure 2). Thus, the cross talk of tyrosine kinases with the DNA Damage Response (DDR) proteins presents a conundrum of oncogene and tumor suppressor interactions and consequently adds complexity into a precisely orchestrated control of genome stability (24). Consequently, deregulated tyrosine kinase driven genomic and epigenomic alterations have profound implications in cancer, aging and genetic disorders. This review discusses novel mechanisms by which tyrosine kinases regulate DDR pathways and the physiological and clinical implications of targeting these pathways in cancer.

Receptor tyrosine kinases are activated by DNA damage

Approximately, 20 receptor tyrosine kinase (RTK) families and at least nine distinct groups of non-receptor tyrosine kinases (NRTK) have been identified in humans, which correspond to about 100 kinases (33). Transmembrane tyrosine kinases, such as the Epidermal Growth Factor Receptor (EGFR) or Insulin like Growth Factor 1 Receptor (IGF-1R) are activated not only in response to extracellular signal originating from the binding of respective ligands or growth factors, but also upon exposure of cells to ionizing radiation, independent of ligand stimulation (Figure 2) (30,34–37). For example, the phosphorylation of EGFR at residues Y845 and Y1173 was found to be strongly induced by ionizing radiation (IR) (38). IR activated EGFR is transported in a Src kinase dependent manner, and interacts with the NHEJ protein, DNAPK (38). EGFR may be also internalized in the nuclear compartment as a component of the lipid rafts or due to its interaction with importin beta (39) (Figure 2). Blocking the nuclear transport of EGFR by Erbitux, markedly impaired radiation associated activation of DNA-PK and increased cellular radio-sensitivity demonstrating a

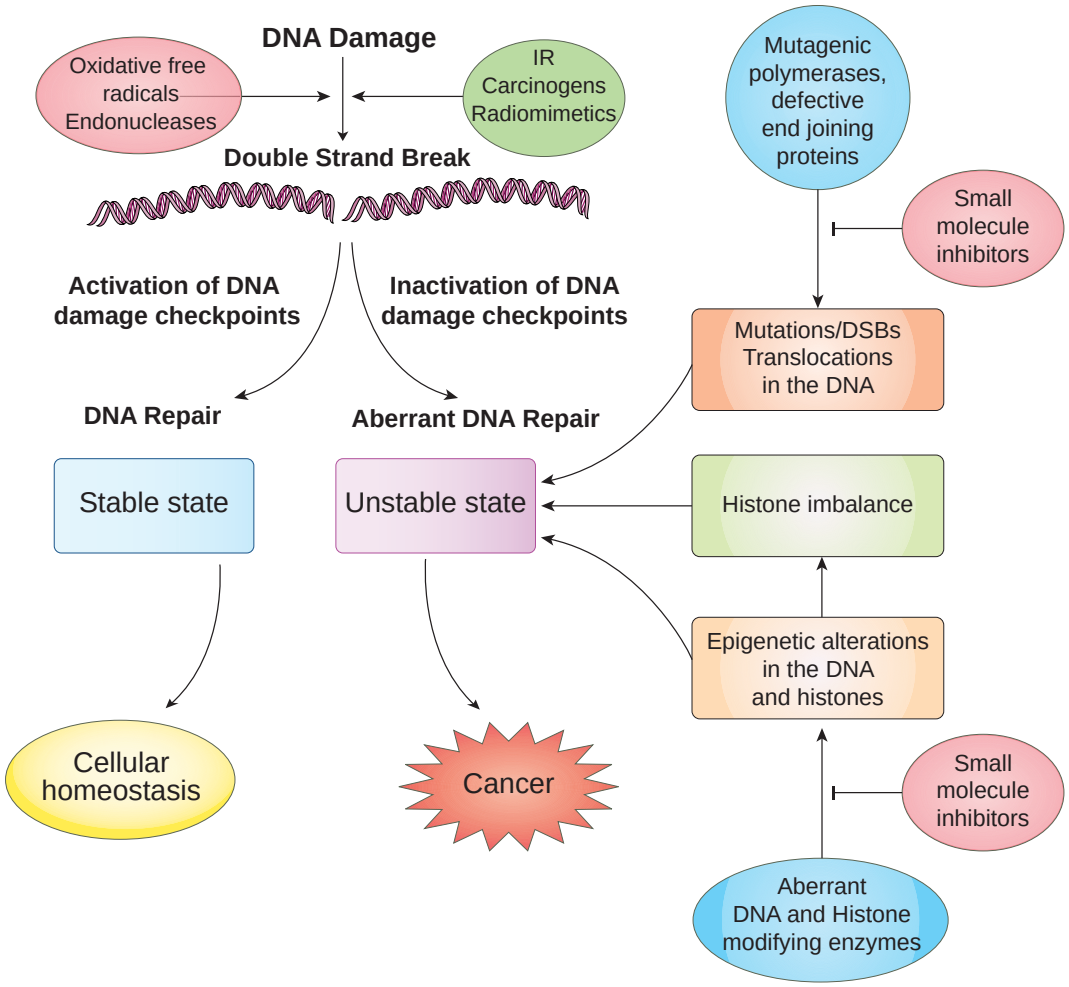


Figure 1. Integrity to the genome is under threat by a variety of exogenous and endogenous agents that activate DNA damage checkpoints. Chromatin alterations can also activate DNA damage signaling pathways. Activated checkpoint kinases, ATM or ATR arrest the cells at a specific stage in the cell cycle and allow time for repair. DNA double strand breaks caused by ionizing radiation may be repaired either by the homologous recombinational repair pathway (HRR) or the non-homologous end joining pathway (NHEJ). In addition, eukaryotic cells face a variety of other situations which could also lead to an unstable genomic state, e.g. mutagenic DNA polymerases, aberrant activity of the end joining proteins, leading to chromosomal translocations, mutations in the DNA and histone modifying enzymes. Small molecule inhibitors targeting these aberrant proteins have emerged to be a therapeutic option which could not only restore genome stability but also inhibit tumor growth by radiosensitization.

direct regulation of NHEJ repair by RTKs (38,40). Moreover, unlike the selectivity seen with the binding of specific growth factors, ionizing radiation randomly activates, albeit to a lesser degree, all members of the EGFR family, ErbB receptors 1–4 (35–37). The random nature of oxidative damage induced by ionization has been suggested as a mechanism for this indiscriminate EGFR family activation (37). This response occurs in a biphasic manner as secondary activation of the EGFR is observed followed initial radiation-induced activation in cancer cells and may be mediated by radiation-induced cleavage and autocrine action of TGFα (41). A proposed mechanism by which EGFR promotes survival in response to radiation in some genetic backgrounds such as K-Ras mutated cell line is the activation of PI3K/AKT signaling pathway that transduces signal to DNAPK stimulating DSB repair (29). Further, treatment of lung cancer cells with EGFR specific small molecule inhibitor gefitinib (Iressa), strongly inhibited the DSB repair after ionizing radiation, indicating that EGFR promotes radiation response by augmenting DNA repair capacity of cancer cells (42).

Ionizing radiation also activates IGF-1R and depletion of IGF-1R or pharmacological blockade of this pathway increased cellular radiosensitivity by modulating DSB repair (30,34) (Figure 2). Additionally, in human neuroblastoma cells, expression of XRCC4, a central component of NHEJ, was significantly upregulated upon overexpression of TrkA receptor tyrosine kinase leading to increased NHEJ activity (43). Consistently, TrKA expression correlated with faster repair of IR-induced DSBs, clinically correlated with increased chromosome stability and favorable prognosis of childhood neuroblastomas. Combined, these studies suggest that activation of tyrosine kinase receptors in response to DNA damage contributes to the genomic stability of cancer cells.

EGFRvIII, a deletion mutant of EGFR that lacks the extracellular, ligand-binding domain, is constitutively active and exhibits higher level of autophosphorylation in re-

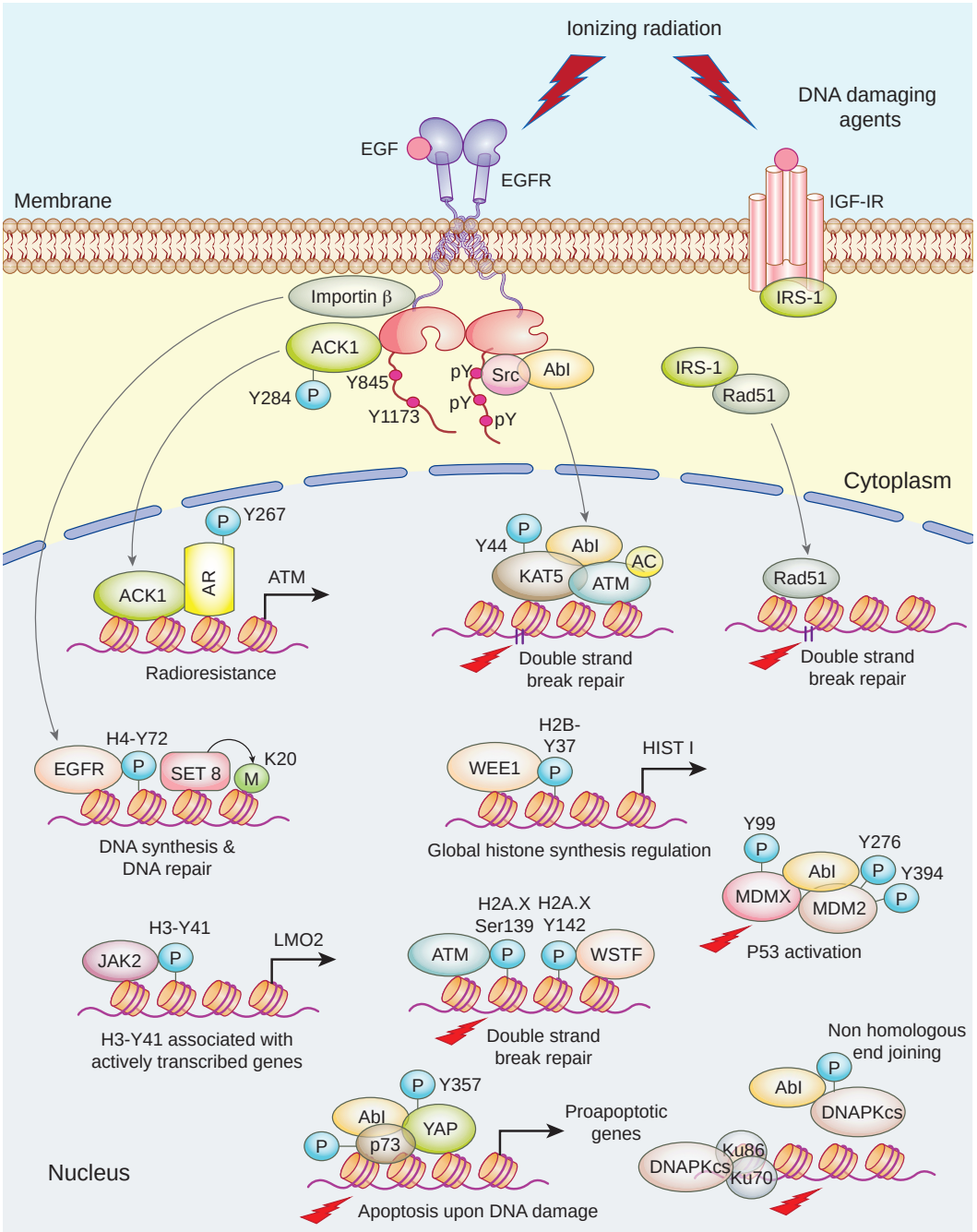


Figure 2. Activation of the DDR proteins in cancerous cells is intricately linked to activated tyrosine kinases and their downstream signaling partners, some of which are also tyrosine kinases. Transmembrane tyrosine kinases, such as the EGFR and IGF-1R are activated by ionizing radiation or by growth factors. Activated EGFR undergoes auto-phosphorylation that in turn recruits and activates non-receptor tyrosine kinases (NRTKs), Src and ACK1/TNK2. These NRTKs shuttle between the cytosol and the nucleus, relaying signals from multiple transmembrane RTKs directly to the nuclear compartment to activate specific DDR pathways. A nuclear form of EGFR modifies, the histone H4 at Tyr72 which functions as a docking site for the chromatin modifying enzymes SET8 and SUV4–20H that methylate H4 lysine 20. In contrast to EGFR, in IR-treated cells, nuclear non-receptor tyrosine kinase Abl directly phosphorylates the histone lysine acetyl transferase KAT5/TIP60 by modifying the Tyr44 in the chromodomain. KAT5 acetylates and activates ATM at DSB sites and loss of KAT5 Tyr44-phosphorylation is associated with increased sensitivity to ionizing radiation. ATM phosphorylates H2A.X at Ser139 residue. In addition, Abl exhibits another response mode- it antagonizes YAP1 oncogenic function by phosphorylating it at Tyr357, which works in conjunction with the tumor suppressor p73 to promote apoptosis in irradiated cells. Abl also antagonizes the negative regulators of the tumor suppressor p53, MDM2 and MDMX, in response to DNA damage. Abl modification of the catalytic subunit, DNAPKCS interferes with the formation of active DNAPK-Ku complex. A non-receptor tyrosine kinase JAK2 utilizes its epigenetic activity wherein it modifies Histone H3 at Tyr41 excluding the binding of heterochromatin protein HP1 which resulted in elevated expression of oncogene *lmo2*. WEE1 has emerged to be another epigenetic tyrosine kinase that directly phosphorylated the histone H2B at Tyr37, consequently terminating global histone transcription at the end of S phase to maintain histone homeostasis. ACK1 is another NRTK that not only phosphorylated Androgen receptor (AR), but also was recruited to a distinct set of cell cycle and DNA damage checkpoint genes, including ATM to confer radioresistance in prostate cancer cells. IGF-IR regulates HRR by modulating the interaction of the insulin receptor substrate 1 (IRS-1) with Rad51.

sponse to IR (44). In contrast to activated EGFR which stimulates both the RAS/RAF/MAPK and PI3K/AKT pathways, EGFRvIII appears to preferentially and robustly activate only the PI3K/AKT signaling cascade (45–47). Consistently, inhibition of PI3K/AKT signaling impaired DSB repair and enhanced radio sensitivity of brain (GBM) as well as breast cancers (48–51). Interestingly, in the light of the recent reports that demonstrate radiation induced AKT translocation to the nucleus and association with DNAPK at DSBs, it is likely that hyperactivation of DNAPK by EGFRvIII/AKT signaling could potentiate DNA repair in cancer cells, opening an entirely new therapeutic options to overcome radiation resistance in cancer cells (52–54). In addition, tyrosine 176 phosphorylated AKT is known to translocate to the nucleus in a PI3K-independent and ACK1 tyrosine kinase dependent manner (55). While activated ACK1 expression is correlated with radioresistance in prostate cancer cells (56), the role of ACK1-pY176AKT signaling in radioresistance is not yet explored.

The internalization of TKs to cues of radiation damage suggests that cells may be using this mechanism to prevent additional growth factor induced signaling cascades until the DNA damage is repaired. Some of studies described below provide further mechanistic insights into how activated oncogenic tyrosine kinases fine tune the repair of radiation induced DNA damage.

Receptor tyrosine kinases epigenetically regulate DDR

Recent studies have uncovered novel epigenetic underpinnings of the RTK-DDR cross talk. The nuclear form of EGFR has been reported to directly modify the core histone H4 at tyrosine 72 (57). The H4 Y72-phosphorylation functions as a docking site for another group of chromatin modifying enzymes SET8 and SUV4-20H that subsequently methylate lysine 20 (Figure 2). Consequently, the H4Y72/H4K20 dual motif directs transcriptional programs for optimal DNA synthesis and DNA repair in cells, implicating RTKs in DNA metabolic activities. Conversely, the H4 Y72 peptide disrupted EGFR-histone H4 interaction and was able to suppress breast cancer xenograft tumor growth (57). With the discovery that the IR-induced checkpoint proteins, mammalian 53BP1 (and its ortholog Crb2 in *S. pombe*), interact with methylated H4K20 at DSB sites via their tudor domains, the loss of H4 Y72-phosphorylation in delaying cell cycle progression could be due to persistent and unrepaired DSBs that eventually lead to apoptosis (58,59). In addition, EGFR is reported to stabilize the binding of the Proliferating Cell Nuclear Antigen (PCNA) to the chromatin by modifying it at Y211 (60). This phosphorylation of PCNA promotes misincorporation of nucleotides during DNA synthesis by inhibiting the activity of mismatch repair proteins at the initiation step (61). Accordingly, interference with the activity of pY211-PCNA led to S-phase arrest, inhibition of DNA synthesis, cytotoxicity and decreased tumor growth of prostate cancer xenograft tumors in mice (62). Collectively these studies emphasize the notion of a direct control of regulation of the DNA damage signaling cascade by RTKs during malignant transformation.

Non-receptor tyrosine kinases regulate DNA damage checkpoint pathways

DDR proteins are also targets of non-receptor tyrosine kinases (NRTKS), such as Abl-1, ACK1 (TNK2) and Src family of tyrosine kinases (Table 2 and Figure 2). Importantly, NRTKs shuttle between the cytosol and the nucleus, and have emerged to be a major intermediary in cellular signaling capable of relaying signals from activated RTKs directly to the nuclear compartment, to activate specific DDR pathways (31,56,63,64).

Abl-1 is a versatile kinase that is activated in cells exposed to genotoxic stress and directly phosphorylates a number of key cellular proteins to promote DNA repair, cell cycle arrest or apoptosis (Table 1 and Figure 2) (65,66). Abl-1 modulates the activity of a number of substrates; Rad51, Rad52, Msh2, ATM, DNAPKCs, BRCA1 and MDMX to regulate some of the major pathways of DNA DSB repair (67,68) (Table 2). Abl-1 also functions as a tumor suppressor in response to radiation induced DNA damage by phosphorylating p73 at Y99 and transactivating and promoting p73 dependent apoptosis (69,70). Recently, it was uncovered that in addition to p73, Abl-1 can also phosphorylate HIPK2 at Tyr360 facilitating p53 dependent apoptosis upon DNA damage (Table 1) (6). Significantly, the DNA damage-induced apoptosis and the activation of the tyrosine kinase c-Abl were regulated by the tumor suppressive Hippo Lats2 kinase depending on the cell density (9). Other mechanisms by which Abl-1 regulates DNA damage checkpoints and DDR proteins to promote genome integrity have been extensively covered (66) and will not be discussed in depth in this article.

In contrast to Abl-1, NRTKs such as ACK1 and the Src family of tyrosine kinases have been shown to regulate radioresistance by indirect mechanisms. ACK1/TNK2 tyrosine phosphorylates AR at Y267 in androgen deprived conditions of prostate cancer cells (56,71,72). Mechanistically, ACK1/pY267-AR complex is recruited to the upstream elements of a distinct set of cell cycle and DNA damage checkpoint genes in an androgen-independent manner (Table 1) (56,72). Notable among these is an upstream binding site in the ATM (Ataxia Telangiectasia Mutated) gene, that contains ARE (androgen responsive elements) sites (56,73). Consequently, activated ACK1 promoted radioresistance of prostate cancer cells and conversely, a small molecule ACK1 inhibitor, e.g. AIM-100 blocks ATM dependent DNA damage induced G2/M arrest, resulting in the accumulation of cytotoxic DSBs (Table 2).

The Src family of NRTKs including Src, Fyn and Lyn may also influence the DDR responses (22,31). Src is known to be phosphorylated upon IR treatment (74). Moreover, the radiation dependent activation of EGFR was found to be Src dependent in some tumor cell lines (38). Intriguingly, the mechanism of action of Src family of kinases appears to be distinct from ACK1, as these kinases oppose the activity of the checkpoint kinases. Just as activation of the DDR pathways is tightly regulated, its deactivation also appears to be precisely controlled within the cells (22). Accordingly, the recovery from G2/M checkpoint arrest via dephosphorylation and degradation of the checkpoint kinases following completion of DNA repair is suggested to be dependent

Table 2. Tyrosine kinases in the DDR network: Alteration frequencies in various cancers

Tyrosine kinase	Alteration frequency	Frequently mutated cancers	Amplification/deletion	Inhibitors/drugs
ABL1	4% SAC, 2% AML	Stomach adenocarcinoma (SAC), Acute myeloid leukemias (AML)	Amplifications and missense mutations, Gene fusion	Imatinib
ACK1 or TNK2	26% LSCC, 27% OSC, 19% HNSCC, 9% SAC, 8% DLBCL	Lung squamous cell carcinoma (LSCC), Ovarian Serous Cystadenocarcinoma (OSC), head and neck squamous cell carcinoma (HNSCC), stomach adenocarcinoma(SAC), diffuse large B cell lymphomas (DLBCL)	Amplifications	AIM-100, (*R*)-9b
EGFR	53% GBM, 8% LSCC, 14%, HNSCC, 11% SAC	Glioblastoma (GBM), lung squamous cell carcinoma (LSCC), head and neck squamous cell carcinoma (HNSCC), stomach adenocarcinoma (SAC)	Amplifications and missense mutations	Gefitinib, Lapatinib, Iressa, Cetuximab/Erbitux, Nimotuzumab
IGF1R	10% SAC, 9% OSC, 7%, LHC, 7% CAC	Stomach adenocarcinoma (SAC), ovarian serous cystadenocarcinoma (OSC), liver hepatocellular carcinoma, colorectal adenocarcinomas (CAC)	Amplifications and missense mutations	Cixutumumab (IMC-A12), Dalotuzumab (MK-0646; h7C10), Linsitinib (OSI-906)
EPHA5	12% LAC, 10% SCLC, 9% SAC,	Lung adenocarcinoma (LAC), small cell lung cancer (SCLC), stomach adenocarcinoma (SAC),	Mutations, amplifications	none
FYN	15% DLBCL	Diffuse large B cell lymphomas (DLBCL)	Deletions	Various Src inhibitors
WEE1	<1%	Glioblastomas, sarcomas	Deletions and missense mutations	AZD1775

The tyrosine kinases that participate in DNA damage response signaling are reported to be frequently mutated in a number of malignancies. The frequency and type of known genetic alteration of the tyrosine kinase, the cancer subtype where it is most frequently mutated and the small molecule inhibitors/drugs targeting each tyrosine kinase signaling pathways are shown.
Reference for mutation frequencies: www.cBioPortal.org.

on the activity of the Src family of tyrosine kinases (31). Although, the exact mechanism is not clear, one putative scenario that has been proposed is the silencing of ATR/Chk1 signaling cascade through an increase in the inhibitory nuclear tyrosine phosphorylation events. These findings may illuminate its well established role as an oncogene wherein it has been demonstrated to have a role in cell proliferation, invasion and motility. In activated Src expressing cancer cells, the cells rapidly recover from stalled replication forks, such as those caused by oncogene-induced replicative stress and resume cell cycle progression (31). Consistent with these observations, Src inhibitors induce a prolonged G2/M arrest and growth inhibition or apoptosis (31). Additionally, the Lyn tyrosine kinase, a member of Src family is activated by ionizing radiation and mitomycin C treatment (75,76) and interacts with the cell division cycle protein Cdc2, DNAPK and protein kinase C delta (PKC delta) in irradiated cells (63,77,78). Thus, the regulation of DDR pathways by oncogenic cytosolic kinases suggests that cancer cells effectively use these survival kinases to circumvent radiation induced apoptosis.

Epigenetic interactions of non-receptor tyrosine kinases with DDR proteins

Cells activate the DDR pathways following exogenous and endogenous DNA damage. However, it is not only a physical damage to the DNA but the chromatin alterations can also trigger DDR pathways. These chromatin alterations can manifest either as structural alterations due to changes in nucleosome packaging, histone imbalance, or due to the removal or addition of specific epigenetic modifications on histones. DNA DSBs induced by the topoisomerase II inhibitor, Adriamycin, can also promote translocation of c-Abl into the nucleus, causing specific hypoacetylation of H4K16 residue in a tyrosine phosphorylation dependent manner (79). Interestingly, hyperacteylation of histones by trichostatin A, a histone deacetylase inhibitor, also activates Abl dependent ATM activation, suggesting that Abl is sensing different epigenetic alterations (10). Further, the bi-directional Abl/ATM cross talk may be a context dependent signaling event that underlies chromatin accessibility at loci with increased H3K9 trimethylation, and is the basis for differences in DDR responses at euchromatin versus heterochromatin loci (10). Notably, increased hyperacetylation and loss of HP1 binding has been shown to augment the Tyr-phosphorylation of histone acetyl transferase, KAT5 (10). The binding of KAT5 to H3K9me3 leads to acetylation and activation of ATM that is needed for Chk2 and p53 phosphorylations to promote DNA damage induced cycle arrest (Table 1). In addition to activating the p53 tumor suppressor dependent apoptotic pathway, c-Abl also promotes p73-YAP dependent apoptosis by negating the oncogenic activity of YAP in response to DNA damage by phosphorylating it at Tyr357 and preventing the activation of the TEAD family of transcription factors (Table 1 and Figure 2) (80). These studies highlight the 'decision maker' function of c-Abl to determine whether to activate apoptotic program or initiate the DNA repair process.

Epigenetic interactions of non-receptor tyrosine kinases with histones to maintain genome integrity

Chromatin duplication is a singularly vulnerable phase in cell cycle. Any incompletely replicated DNA or excess histones can perturb histone/DNA stoichiometry, disrupt chromosome homeostasis to promote genome instability and mitotic catastrophe. Additionally, histone imbalance can alter stable gene expression profiles in cells by inducing epigenetic alterations that are often linked to cancer and developmental disorders (81–86). There are at least two examples by which tyrosine kinase and proteins with tyrosine kinase like activity maintain histone/DNA stoichiometry of the chromatin. The first example is the DNA damage checkpoint kinase from the budding yeast, *Saccharomyces cerevisiae*, Rad53, (homolog of CHK2 in humans) which regu-

lates histone homeostasis within cells (87). Rad53 harbors both serine/threonine and tyrosine kinase like-activity and protects cells from cytotoxic effect of excess histones. Mechanistically, it phosphorylates excess non-chromatin bound histone H3 at Tyr99 and flags it for proteosomal degradation (88,89). Importantly, its ability to prevent excess non-nucleosomal histones from interfering with the initiation of DNA replication was found to be independent of its role as an effector of the Mec1 mediated replication and DNA damage checkpoint activity in stabilizing stalled replication forks (90). Consistently, deletion of a major copy of the H3-H4 genes in *S. cerevisiae* suppressed the synthetic lethality associated with replication defective mutant of Rad53 (*rad53-rep*) and the cell division cycle protein 7 *(cdc7-1)* (homolog of CDC7Hs in humans). Together, these studies provide insights into how tyrosine and threonine kinase activity of Rad53 may be employed to regulate two distinct protein–protein interaction networks within cells (90,91).

The second example is WEE1 kinase, which directly phosphorylates the histone H2B at Tyr37, to terminate global histone transcription at the end of S phase (92). Mechanistically, the histone transcriptional repressor, HIRA acts as the 'reader' of the pY37-H2B epigenetic marks and its recruitment excluded the histone transcriptional co-activator, NPAT, to suppress global histone mRNA synthesis. Importantly, the WEE1-specific small molecule inhibitor, MK-1775 (also known as AZD1775), reversed histone transcriptional suppression by blocking this WEE1 epigenetic function (Table 1 and Figure 2). The role of H2B Tyr37-phosphorylation in G2/M arrest has not been elucidated so far. Future studies investigating its requirement in IR-induced or replication stalling induced WEE1 checkpoint functions may reveal whether anti-tumor activity of WEE1 inhibitors is a combination of suppression of WEE1 epigenetic activity and blocade of cell cycle progression.

JAK2, another NRTK, phosphorylates histone H3 at tyrosine 41 to regulate transcription by marking several active promoters, including the leukeomogenic oncogene Imo2, which regulates hematopoietic stem cell development in normal cells (93). Increased levels of phosphorylated H3Y41 as well as JAK2 activity is seen with GBMs and treatment with JAK2 inhibitors was found to overcome radioresistance of GBMs treated with temozolomide and IR (94). Overall, the ability of NRTKs to interact directly with the chromatin in normal and cancer cells has opened doors to further interrogate their direct roles in regulating DNA damage signaling and chromatin integrity in response to radio- and chemotherapy.

Tyrosine kinases in DSB repair

Tyrosine kinases also influence DNA DSB repair by directly modifying proteins involved in HR or NHEJ following DNA damage. EGFR/AKT/ERK signaling is activated and protects tumor cells from cytotoxic doses of ionizing radiation induced DNA damage (35,37,41). In addition, somatic mutations in EGFR tyrosine kinase have been shown to differentially modulate DNA double strand break repair as seen by differences in the kinetics of resolution of IR induced H2AX foci and enhanced clonogenic survival compared to the wild type EGFR (35,37,41). In addition to EGFR, IGF-1R also regulates DSB repair mediated by both, non-homologous end-joining and homologous recombinational repair proteins (HR) (30,34,95–97). Mechanistically, IGF-IR tyrosine phosphorylates a major downstream substrate, the insulin receptor substrate 1 (IRS-1) upon IGF-1 stimulation (30) IRS-1 interaction with Rad51 is negatively regulated by tyrosine phosphorylation in the cytosol. IGF-1 stimulation disrupts the IRS-1-Rad51 complex promoting Rad51 entry into the nucleus (Figure 2). Conversely, attenuation of IGF-1R signaling lead to diminished translocation of Rad51 to the site of damaged DNA, compromising homologous recombination-directed DNA repair (Table 2) (30). In contrast in lung cancer cells, IGF-1R inhibition decreased the radiation-induced Ku-DNA-binding in a p38 MAPK dependent manner supporting a role for IGF-1R signaling in NHEJ mediated DSB repair (34).

Although the exact mechanism is not known, expression of TrkA tyrosine kinases accelerates IR induced DNA DSB repair by upregulating the expression of the NHEJ protein XRCC4 in neuroblastoma cell lines (43). Similarly, fusion tyrosine kinases drive drug resistance in hematopoietic cells by utilizing multiple routes *i.e.* upregulating STAT5 dependent upregulation of RAD51, recruiting it to repair DSBs by homologous recombination, promoting anti-apoptotic activity of BCL-XL and delaying cell cycle progression (98).

The instantaneous activation of c-Abl kinase upon IR-induced damage is primarily mediated by ATM via phosphorylation at Ser465 or may be mediated by DNAPK at an unknown site (65,99,100). Intriguingly, this DDR-TK regulation appears to be bidirectional, as ATM activation is itself regulated indirectly by c-Abl activity when complexed with KAT5/TIP60 (10). In IR-treated cells, Abl phosphorylates the histone lysine acetyl transferase KAT5/TIP60 by modifying the Tyr44 in the chromodomain (Table 1). Loss of KAT5 Tyr44-phosphorylation is associated with increased sensitivity to ionizing radiation. ATM and KAT5 form a distinct complex (101) that is recruited near DSB sites, displacing the heterochromatin protein 1 (HP1) at H3K9me3 in a MRN (Mre11-Rad50-Nbs1) dependent manner (102). Abl is acetylated by TIP60/KAT5 in response to DSBs at K921 which is required for IR induced apoptosis (103). c-Abl has also been shown to directly interact with and phosphorylate DNAPK, causing the dissociation of DNAPK/Ku protein complex from the DNA and attenuating NHEJ repair (104). Interestingly, in cells exposed to IR, c-Abl/BRCA1 interaction is also compromised (105). Further, c-Abl activated by IR was also shown to mediate phosphorylation of PI3K and mTOR, causing the inhibition their kinase activity (106,107). IR activated c-Abl tyrosine phosphorylates Rad51 at Tyr315 which increases association with Rad52 and chromatin in the recombination complex, in an ATM dependent manner (108,109). Interestingly, Rad52 is also phosphorylated at Tyr104 and this modification is required for the formation of ionizing radiation induced foci (IRIF) (110,111). These results suggest that Abl-1 is a critical regulator of DNA damage responses by post-translationally modifying epigenetic and non-epigenetic regulators to promote DNA repair or apoptosis.

An off-on tyrosine/serine phosphorylation switch in the tail of variant histone, Histone H2A.X, directs repair in response to DSBs (112,113). ATM phosphorylates the histone variant, H2A.X, at Serine 139 (γH2A.X) in response to DNA DSBs. γH2A.X functions as an active epigenetic mark for the recruitment of the mediator/adaptor proteins and assembly of multimeric protein complexes critical for DNA damage signaling and repair (114). Interestingly, H2AX is modified constitutively at Tyr142 phosphorylation by a non-canonical tyrosine kinase, WSTF (William-Beuren Syndrome Transcription Factor) (Table 1 and Figure 2) (112). These pY142 H2A.X marks are progressively erased following DNA damage and are suggested to be important to direct cells to choose repair or apoptosis (113–115). Intriguingly, mutation in the Y142 sensitizes chicken DT-40 cells to IR-induced DNA damage, suggesting that in wild-type cells, Tyr142-phosphorylation co-ordinates with increased Ser139-phosphorylation to facilitate DNA repair, before its complete removal on the chromatin. MCPH1, a tandem BRCT domain containing protein was found to specifically interact with the diphosphorylated-H2A.X (113–115). Paradoxically, a complete loss of both Ser139 and Tyr142 phosphorylations at the C-terminal tail of H2A.X appears to alleviate the radiation sensitivity of the Y142 mutant alone. The observation that another tandem BRCT domain protein, 53BP1, previously shown to colocalize with γH2A.X at damage sites, persisted at IRIFs in the H2AX-Ser139/Tyr142 double mutants, suggested that assembly at DNA ends may be compensated by BRCT domain containing proteins that are recruited through other histone modifications at the DSBs. In one example, MMSET a histone methyltransferase was found to interact with the BRCT domain of MDC1, an adaptor protein that binds to phosphoS139-H2A.X. Subsequently, MMSET methylates H4K20 locally at DSB sites and this methylation is critical to recruit 53BP1 (59). However, MDC1 recruitment is dependent on H2AX S139 phosphorylation. Therefore, identification of other histone modifications or chromatin regulators that can recruit MDC1 in the absence of H2AX phosphorylation would be the key to identify alternative mode of assembly of checkpoint proteins and DNA repair factors at DSB sites. Indeed, a recent report has identified a role of H2A.X methylation at lysine 134 in regulating γH2A.X levels in cancer cells, loss of which resulted in severe sensitivity to IR, and DSB inducing chemotherapeutic agents, Cisplatin and Doxorubicin (116).

A chromatin interacting protein, localized to DNA lesions is the hPso4/Prp19, which was originally identified for its ability to interact with the BRCT domain of the template independent DNA polymerase, terminal deoxynucleotidyl transferase (TdT), but has subsequently been shown to be phosphorylated by ATM following DNA damage (117,118). Importantly, it is upregulated in response to high doses of radiation and its interaction with chromatin is enhanced in the presence of DNA damage. Pso4 localizes with Nbs1 and Metnase at DSBs, and promotes DNA end-joining following IR induced DNA damage (117,119). Conversely, cells with loss of Pso4 protein display sensitivity to DSB inducing agents (117,120). Whether Pso4 interacts with the BRCT domain of MDC1 at DSB sites to promote alternative NHEJ repair remains to be seen.

A role for the Src family of kinases, Src, Fyn, and Abl kinases, in causing global nuclear structural changes by altering chromatin has been uncovered by many groups. These kinases regulate the interaction of Protein kinase A anchoring protein 8 (AKAP8, also known as AKAP95) with the chromatin and the nuclear matrix by modifying it by tyrosine phosphorylation (121). AKAP8/AKAP95 is a zinc finger containing protein with RNA binding activity (122) that interacts with the RII-alpha regulatory subunit of protein kinase A (PKA) and implicated in chromosome condensation during mitosis (123). Interestingly, similar to ACK1, Src is also known to interact and modify AR to modulate expression of the AR target genes in prostate cancer cell lines (124). Whether tyrosine phosphorylated Src can directly regulate DDR is not known, nor is it clear whether it would modulate KDM3A or other related chromatin modifiers, particularly under conditions where ACK1 is inhibited. However, its ability to shuttle between the cytosol and nucleus is suggestive of this possibility. Collectively, these studies collectively paint a picture of the involvement of NRTKs in direct interactions with DDR in adverse treatment-rich environment to promote hormonally regulated cancers.

Physiological and clinical relevance of DDR and TK crosstalk

The physiological significance of tyrosine kinase interaction with DDR pathways is still fairly underexplored. Mice with genetic deficiency in nuclear TK, WEE1 or in the DNA repair proteins, Rad51 and PSO4/PRP19 are embryonic lethal, suggesting that some of these interactions are essential for early development (125–128). Despite the inherent genome instability within cancer cells, oncogenic tyrosine kinases employ the DNA damage response (DDR) pathways to bypass checkpoints in conjunction with activating other survival pathways promoting tumor growth. Cross-cancer alteration analysis for Abl-1, EGFR, Fyn, LCK, Src, ACK1 (TNK2) tyrosine kinases reveal that these genes are frequently altered in cancers, e.g. EGFR and TNK2 are recurrently amplified and mutually exclusive in a majority of the cancer cases (Table 2). This table not only details the alteration in those tyrosine kinases that are involved in DDR, but also shows that many of the tyrosine kinase inhibitors or TKIs have been employed in clinical setting as cancer drugs. Not surprisingly, TKIs have emerged as one of the most common therapeutic strategy for the treatment of a variety of cancers, either alone (1–4) or in combination with radiotherapy (23). Consistently, EGFR activation is associated with tumor radioresistance and poor prognosis in GBMs and lung cancers (35,37,41). Conversely, blockade of EGFR/AKT/ERK signaling with small molecule inhibitors compromised DSB repair by blocking NHEJ and HRR. Certain cancers associated with activating mutations of EGFR also appear to influence phosphorylation and expression of members of DDR response pathways, including phospho-DNAPK, ATM, and RAD51 foci, suggesting that both HRR and NHEJ pathways may have roles in conferring radioresistance.

Frequently considered are the TKIs, gefitinib, lapatinib and erlotinib to block EGFR, as activation of this tyro-

sine kinase was found to correlate with radioresistance and survival. Gefitinib and lapatinib have been reported to inhibit DNA DSB repair in HER2 amplified breast cancer cell lines (129). High expression of RTKs, e.g. EGFR expression could lead to a higher degree of radiosensitization with erlotinib when combined with radiation (130). Further, EGFR inhibitors or blockade of the EGFR protein with selective monoclonal antibodies sensitized cancer cells to radiation therapy by blocking the repair of damaged DNA (39) and improved clinical outcomes in a subset of patients with head and neck squamous cell carcinoma (131). The molecular mechanisms underlying radiosensitization of cancer cells following EGFR inhibition is not completely clear, as it varies with the tissue of origin. Multiple mechanisms have been explored, for example, it has been shown that the radiosensitization by lapatinib is primarily mediated through inhibition of RAF/MEK/ERK pathway and combinatorial approach that also includes inhibitors of this pathway could provide superior strategy for radiosensitization in HER2-positive breast cancers (132). Other mechanisms included inhibition of EGFR nuclear transport to prevent interaction with DNAPK and inhibition of the PI3K-AKT pathway (133,134). However, not all combinatorial approaches lead to successes. Cetuximab is often combined with radiotherapy in advanced squamous cell carcinoma of the head and neck (SCCHN), but the inhibition of Src kinase by Dasatinib did not improve the efficacy of Cetuximab combined with radiotherapy (135).

BCR-ABL oncogenic fusion tyrosine kinase, mutated in 100% of CMLs and a subset of ALLs was also found to have a differential effect on the repair of DNA damage caused by various genotoxic agents. It increased the efficiency of repair in response to DNA strand breaks in BaF3 lymphoid cells, e.g. Idarubicin induced DSBs were efficiently repaired in BCR-ABL-positive cells suggesting involvement of this mutant kinase in their resistance to Idarubicin (136). However, BCR-ABL expressing CMLs develop resistance to the selective inhibitors such as Imatinib and may rely on the alt-NHEJ pathway for repair. Consistently targeting Parp1 a member of alt-NHEJ with selective inhibitors sensitized TKI resistant BCR-ABL CMLs (137). Additionally BCR-ABL, differentially affected nucleotide excision repair in response to UV-C induced DNA damage based on the source of origin of the cell type, i.e. lymphoid vs myeloid (138). While the p210-BCR-ABL suppressed nucleotide excision repair (NER) in the BaF3 lymphoid background and correspondingly increased the mutation rates following UV-C induced damage, it conferred resistance to UV radiation in myeloid cells and primary bone marrow cells by increasing the NER activity. The UV resistance could be overcome by treatment with the selective TKI inhibitor, ST1571 (137). These observations suggest that although the oncogenic kinases are the main drivers of malignancy, their outcomes on DNA repair is influenced by additional factors.

TKIs have also been employed based on their ability to differentially suppress either the homologous recombination or the non-homologous pathway of endjoining in specific cell types and thus shown to have a therapeutic benefit. Some cell lines that mimicked the invasive bladder tumors were observed to be defective in non-homologous endjoining (NHEJ). Mechanistically, Imatinib, a BCR-ABL inhibitor has an apparent inhibitory effect on the function of the HR protein, RAD51 (139). These cell lines were treated with a combination of radiotherapy and the small molecule tyrosine kinase inhibitor imatinib, based on the premise that inhibiting both HR and NHEJ is likely to induce toxicity. In another study, Sorafenib, a potent inhibitor of Raf kinase and VEGF receptor, significantly enhanced the antiproliferative effects of chemoradiation treatment by downregulating DNA repair proteins (ERCC-1 and XRCC-1) in a dose-dependent manner (140).

Similar to many oncogenic TKs, ACK1 activation too is linked to poor prognosis in a number of cancers (141) (Table 2). ACK1 tyrosine kinase not only interacts with AR in prostate cancer (Table 1) (72), but has also been shown to interact with estrogen receptor (ER) in breast cancer cells (142). Significantly, ACK1 phosphorylated the ER co-activator, KDM3A, a H3K9 demethylase, at an evolutionary conserved tyrosine 1114 site in the presence of tamoxifen, an ER antagonist (Table 1). Not surprisingly, ACK1 activation resulted in a significant decrease in the deposition of dimethyl H3K9 epigenetic marks, while its inhibition restored the repressive marks and caused transcriptional suppression of the ER-regulated genes such as the mammary tumor oncogene, *HOXA1*. Although AR is known to interact with KDM3A (143,144), whether ACK1/AR signaling utilizes KDM3A to erase the repressive H3K9 methylation marks to activate ATM expression to promote radioresistance of castration resistant prostate cancer is unknown.

Glioblastomas (GBMs) are incurable malignancies and highly resistant to radio- and chemotherapy. WEE1 and JAK2 tyrosine kinases are either upregulated or highly active in GBMs and correlated with poor prognosis (94,145). Radioresistance of GBMs is subjugated by treatment with the WEE1 inhibitor, AZD1775 (146), or the Jak2 inhibitor, AZD1480. AZD1775 is currently in clinical trials, primarily due to its ability to avert the G2/M checkpoint arrest by abrogating Cdc2Y15 phosphorylation and induce mitotic catastrophe in irradiated cancer cells with defective p53 (147) (Tables 1 and 2). Since these kinases have non-overlapping roles in conferring radioresistance, it remains to be seen whether combination of these two inhibitors can lead to better therapeutic outcomes.

CONCLUSION

All the above examples, unique as they may be, illustrate the critical contribution of tyrosine kinase family of proteins in promoting DNA repair, cell cycle progression, apoptosis and maintenance of epigenetic states-processes that are fundamental to cellular homeostasis. Recent studies unraveling the evolutionary dynamics of cancer metastasis using androgen-deprived prostate cancer as a model however reinforce the requirement for a deeper understanding of the mechanics of proteins and regulators critical for the maintenance of genome stability. Genes frequently perturbed in the metastatic sub clones were those associated with the DNA repair genes, including mutations in the hypermutator mismatch repair gene, MSH2, the DNA polymerase polE and DNA repair gene *polD1*, the homologous recombination repair genes RAD52, BRCA2 and ATM, as well as missense mutations in the tumor suppressor p53 (148). AR

amplification was also frequently observed in metastatic prostate cancer. AR promotes radioresistance of prostate cancers using androgen dependent or androgen independent but TK dependent mechanisms (56,149). As summarized above, many of these gene products are modified by oncogenic tyrosine kinases. Future studies exploring the cross talk of tyrosine kinases and DDR pathways during the evolutionary progression of aggressive cancers to therapy resistance and metastasis should yield a better understanding of the precise role of founder mutations and secondary mutations. Further, these studies will provide suitable rationale for designing novel targeted therapies.

Department of Defense awards [W81XWH-12-1-0248, W81XWH-14-1-0251 and W81XWH-15-1-0059 to K.M.] (in part); National Cancer Institute, NIH [1R01CA135328]; Department of Defense [W81XWH-14-1-0002, W81XWH-14-1-0003 and W81XWH-15-1-0311]; Miles for Moffitt grants (to N.P.M.). Funding for open access charge: Moffitt Cancer Center.

Conflict of interest statement. None declared.

REFERENCES

1. Krause,D.S. and Etten,R.A. (2005) Tyrosine kinases as targets for cancer therapy. *N. Engl. J. Med.*, **353**, 172–187.
2. Hartmann,J.T., Haap,M., Kopp,H.G. and Lipp,H.P. (2009) Tyrosine kinase inhibitors – a review on pharmacology, metabolism and side effects. *Curr. Drug Metab.*, **10**, 470–481.
3. Arora,A. and Scholar,E.M. (2005) Role of tyrosine kinase inhibitors in cancer therapy. *J. Pharmacol. Exp. Ther.*, **315**, 971–979.
4. O'Shea,J.J., Holland,S.M. and Staudt,L.M. (2013) JAKs and STATs in immunity, immunodeficiency, and cancer. *N. Engl. J. Med.*, **368**, 161–170.
5. Tu,C.C., Zhong,Y., Nguyen,L., Tsai,A., Sridevi,P., Tarn,W.Y. and Wang,J.Y. (2015) The kinase ABL phosphorylates the microprocessor subunit DGCR8 to stimulate primary microRNA processing in response to DNA damage. *Sci. Signal.*, **8**, ra64.
6. Reuven,N., Adler,J., Porat,Z., Polonio-Vallon,T., Hofmann,T.G. and Shaul,Y. (2015) The tyrosine kinase c-Abl promotes homeodomain-interacting protein kinase 2 (HIPK2) accumulation and activation in response to DNA damage. *J. Biol. Chem.*, **290**, 16478–16488.
7. Staquicini,F.I., Qian,M.D., Salameh,A., Dobroff,A.S., Edwards,J.K., Cimino,D.F., Moeller,B.J., Kelly,P., Nunez,M.I., Tang,X. *et al.* (2015) Receptor tyrosine kinase EphA5 is a functional molecular target in human lung cancer. *J. Biol. Chem.*, **290**, 7345–7359.
8. Chen,E., Ahn,J.S., Massie,C.E., Clynes,D., Godfrey,A.L., Li,J., Park,H.J., Nangalia,J., Silber,Y., Mullally,A. *et al.* (2014) JAK2V617F promotes replication fork stalling with disease-restricted impairment of the intra-S checkpoint response. *Proc. Natl. Acad. Sci. U.S.A.*, **111**, 15190–15195.
9. Reuven,N., Adler,J., Meltser,V. and Shaul,Y. (2013) The Hippo pathway kinase Lats2 prevents DNA damage-induced apoptosis through inhibition of the tyrosine kinase c-Abl. *Cell Death Differ.*, **20**, 1330–1340.
10. Kaidi,A. and Jackson,S.P. (2013) KAT5 tyrosine phosphorylation couples chromatin sensing to ATM signalling. *Nature*, **498**, 70–74.
11. Kondratova,A., Watanabe,T., Marotta,M., Cannon,M., Segall,A.M., Serre,D. and Tanaka,H. (2015) Replication fork integrity and intra-S phase checkpoint suppress gene amplification. *Nucleic Acids Res*, **43**, 2678–2690.
12. Ghezraoui,H., Piganeau,M., Renouf,B., Renaud,J.B., Sallmyr,A., Ruis,B., Oh,S., Tomkinson,A.E., Hendrickson,E.A., Giovannangeli,C. *et al.* (2014) Chromosomal translocations in human cells are generated by canonical nonhomologous end-joining. *Mol. Cell*, **55**, 829–842.
13. Richardson,C. and Jasin,M. (2000) Frequent chromosomal translocations induced by DNA double-strand breaks. *Nature*, **405**, 697–700.
14. Wray,J., Williamson,E.A., Singh,S.B., Wu,Y., Cogle,C.R., Weinstock,D.M., Zhang,Y., Lee,S.H., Zhou,D., Shao,L. *et al.* (2013) PARP1 is required for chromosomal translocations. *Blood*, **121**, 4359–4365.
15. Li,X. and Heyer,W.D. (2008) Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res.*, **18**, 99–113.
16. Lieber,M.R., Gu,J., Lu,H., Shimazaki,N. and Tsai,A.G. (2010) Nonhomologous DNA end joining (NHEJ) and chromosomal translocations in humans. *Sub-cell. Biochem.*, **50**, 279–296.
17. Fattah,F., Lee,E.H., Weisensel,N., Wang,Y., Lichter,N. and Hendrickson,E.A. (2010) Ku regulates the non-homologous end joining pathway choice of DNA double-strand break repair in human somatic cells. *PLoS Genet.*, **6**, e1000855.
18. Roberts,S.A., Strande,N., Burkhalter,M.D., Strom,C., Havener,J.M., Hasty,P. and Ramsden,D.A. (2010) Ku is a 5′-dRP/AP lyase that excises nucleotide damage near broken ends. *Nature*, **464**, 1214–1217.
19. Bunting,S.F. and Nussenzweig,A. (2013) End-joining, translocations and cancer. *Nat. Rev. Cancer*, **13**, 443–454.
20. O'Rourke,D.M., Kao,G.D., Singh,N., Park,B.W., Muschel,R.J., Wu,C.J. and Greene,M.I. (1998) Conversion of a radioresistant phenotype to a more sensitive one by disabling erbB receptor signaling in human cancer cells. *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 10842–10847.
21. De Bacco,F., Luraghi,P., Medico,E., Reato,G., Girolami,F., Perera,T., Gabriele,P., Comoglio,P.M. and Boccaccio,C. (2011) Induction of MET by ionizing radiation and its role in radioresistance and invasive growth of cancer. *J. Natl. Cancer Inst.*, **103**, 645–661.
22. Shields,B.J., Hauser,C., Bukczynska,P.E., Court,N.W. and Tiganis,T. (2008) DNA replication stalling attenuates tyrosine kinase signaling to suppress S phase progression. *Cancer Cell*, **14**, 166–179.
23. Valerie,K., Yacoub,A., Hagan,M.P., Curiel,D.T., Fisher,P.B., Grant,S. and Dent,P. (2007) Radiation-induced cell signaling: inside-out and outside-in. *Mol. Cancer Ther.*, **6**, 789–801.
24. Motoyama,N. and Naka,K. (2004) DNA damage tumor suppressor genes and genomic instability. *Curr. Opin. Genet. Dev.*, **14**, 11–16.
25. Gire,V., Roux,P., Wynford-Thomas,D., Brondello,J.M. and Dulic,V. (2004) DNA damage checkpoint kinase Chk2 triggers replicative senescence. *EMBO J.* **23**, 2554–2563.
26. Hayashi,M.T., Cesare,A.J., Rivera,T. and Karlseder,J. (2015) Cell death during crisis is mediated by mitotic telomere deprotection. *Nature*, **522**, 492–496.
27. Herbig,U., Jobling,W.A., Chen,B.P., Chen,D.J. and Sedivy,J.M. (2004) Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol. Cell*, **14**, 501–513.
28. Liberal,V., Martinsson-Ahlzen,H.S., Liberal,J., Spruck,C.H., Widschwendter,M., McGowan,C.H. and Reed,S.I. (2012) Cyclin-dependent kinase subunit (Cks) 1 or Cks2 overexpression overrides the DNA damage response barrier triggered by activated oncoproteins. *Proc. Natl. Acad. Sci. U.S.A.*, **109**, 2754–2759.
29. Toulany,M., Kasten-Pisula,U., Brammer,I., Wang,S., Chen,J., Dittmann,K., Baumann,M., Dikomey,E. and Rodemann,H.P. (2006) Blockage of epidermal growth factor receptor-phosphatidylinositol 3-kinase-AKT signaling increases radiosensitivity of K-RAS mutated human tumor cells in vitro by affecting DNA repair. *Clin. Cancer Res.*, **12**, 4119–4126.
30. Trojanek,J., Ho,T., Del Valle,L., Nowicki,M., Wang,J.Y., Lassak,A., Peruzzi,F., Khalili,K., Skorski,T. and Reiss,K. (2003) Role of the insulin-like growth factor I/insulin receptor substrate 1 axis in Rad51 trafficking and DNA repair by homologous recombination. *Mol. Cell. Biol.*, **23**, 7510–7524.
31. Fukumoto,Y., Morii,M., Miura,T., Kubota,S., Ishibashi,K., Honda,T., Okamoto,A., Yamaguchi,N., Iwama,A., Nakayama,Y. *et al.* (2014) Src family kinases promote silencing of ATR-Chk1 signaling in termination of DNA damage checkpoint. *J. Bol. Chem.*, **289**, 12313–12329.
32. Fukumoto,Y., Kuki,K., Morii,M., Miura,T., Honda,T., Ishibashi,K., Hasegawa,H., Kubota,S., Ide,Y., Yamaguchi,N. *et al.*

(2014) Lyn tyrosine kinase promotes silencing of ATM-dependent checkpoint signaling during recovery from DNA double-strand breaks. *Biochem. Biophys. Res. Commun.*, **452**, 542–547.
33. Manning,G., Whyte,D.B., Martinez,R., Hunter,T. and Sudarsanam,S. (2002) The protein kinase complement of the human genome. *Science (New York, N.Y.)*, **298**, 1912–1934.
34. Cosaceanu,D., Budiu,R.A., Carapancea,M., Castro,J., Lewensohn,R. and Dricu,A. (2007) Ionizing radiation activates IGF-1R triggering a cytoprotective signaling by interfering with Ku-DNA binding and by modulating Ku86 expression via a p38 kinase-dependent mechanism. *Oncogene*, **26**, 2423–2434.
35. Schmidt-Ullrich,R.K., Mikkelsen,R.B., Dent,P., Todd,D.G., Valerie,K., Kavanagh,B.D., Contessa,J.N., Rorrer,W.K. and Chen,P.B. (1997) Radiation-induced proliferation of the human A431 squamous carcinoma cells is dependent on EGFR tyrosine phosphorylation. *Oncogene*, **15**, 1191–1197.
36. Goldkorn,T., Balaban,N., Shannon,M. and Matsukuma,K. (1997) EGF receptor phosphorylation is affected by ionizing radiation. *Biochim. Biophys. Acta*, **1358**, 289–299.
37. Bowers,G., Reardon,D., Hewitt,T., Dent,P., Mikkelsen,R.B., Valerie,K., Lammering,G., Amir,C. and Schmidt-Ullrich,R.K. (2001) The relative role of ErbB1–4 receptor tyrosine kinases in radiation signal transduction responses of human carcinoma cells. *Oncogene*, **20**, 1388–1397.
38. Dittmann,K., Mayer,C., Kehlbach,R. and Rodemann,H.P. (2008) Radiation-induced caveolin-1 associated EGFR internalization is linked with nuclear EGFR transport and activation of DNA-PK. *Mol. Cancer*, **7**, 69.
39. Teng,K., Zhang,Y., Hu,X., Ding,Y., Gong,R. and Liu,L. (2015) Nimotuzumab enhances radiation sensitivity of NSCLC H292 cells in vitro by blocking epidermal growth factor receptor nuclear translocation and inhibiting radiation-induced DNA damage repair. *OncoTargets Ther.*, **8**, 809–818.
40. Dittmann,K., Mayer,C. and Rodemann,H.P. (2005) Inhibition of radiation-induced EGFR nuclear import by C225 (Cetuximab) suppresses DNA-PK activity. *Radiother. Oncol.*, **76**, 157–161.
41. Dent,P., Reardon,D.B., Park,J.S., Bowers,G., Logsdon,C., Valerie,K. and Schmidt-Ullrich,R. (1999) Radiation-induced release of transforming growth factor alpha activates the epidermal growth factor receptor and mitogen-activated protein kinase pathway in carcinoma cells, leading to increased proliferation and protection from radiation-induced cell death. *Mol. Biol. Cell*, **10**, 2493–2506.
42. Tanaka,T., Munshi,A., Brooks,C., Liu,J., Hobbs,M.L. and Meyn,R.E. (2008) Gefitinib radiosensitizes non-small cell lung cancer cells by suppressing cellular DNA repair capacity. *Clin. Cancer Res.*, **14**, 1266–1273.
43. Schulte,J.H., Kuhfittig-Kulle,S., Klein-Hitpass,L., Schramm,A., Biard,D.S., Pfeiffer,P. and Eggert,A. (2008) Expression of the TrkA or TrkB receptor tyrosine kinase alters the double-strand break (DSB) repair capacity of SY5Y neuroblastoma cells. *DNA Repair*, **7**, 1757–1764.
44. Lammering,G., Hewit,T.H., Valerie,K., Contessa,J.N., Amorino,G.P., Dent,P. and Schmidt-Ullrich,R.K. (2003) EGFRvIII-mediated radioresistance through a strong cytoprotective response. *Oncogene*, **22**, 5545–5553.
45. Learn,C.A., Hartzell,T.L., Wikstrand,C.J., Archer,G.E., Rich,J.N., Friedman,A.H., Friedman,H.S., Bigner,D.D. and Sampson,J.H. (2004) Resistance to tyrosine kinase inhibition by mutant epidermal growth factor receptor variant III contributes to the neoplastic phenotype of glioblastoma multiforme. *Clin. Cancer Res.*, **10**, 3216–3224.
46. Li,B., Yuan,M., Kim,I.A., Chang,C.M., Bernhard,E.J. and Shu,H.K. (2004) Mutant epidermal growth factor receptor displays increased signaling through the phosphatidylinositol-3 kinase/AKT pathway and promotes radioresistance in cells of astrocytic origin. *Oncogene*, **23**, 4594–4602.
47. Golding,S.E., Morgan,R.N., Adams,B.R., Hawkins,A.J., Povirk,L.F. and Valerie,K. (2009) Pro-survival AKT and ERK signaling from EGFR and mutant EGFRvIII enhances DNA double-strand break repair in human glioma cells. *Cancer Biol. Ther.*, **8**, 730–738.
48. Kao,G.D., Jiang,Z., Fernandes,A.M., Gupta,A.K. and Maity,A. (2007) Inhibition of phosphatidylinositol-3-OH kinase/Akt signaling impairs DNA repair in glioblastoma cells following ionizing radiation. *J. Biol. Chem.*, **282**, 21206–21212.
49. Mukherjee,B., McEllin,B., Camacho,C.V., Tomimatsu,N., Sirasanagandala,S., Nannepaga,S., Hatanpaa,K.J., Mickey,B., Madden,C., Maher,E. *et al.* (2009) EGFRvIII and DNA double-strand break repair: a molecular mechanism for radioresistance in glioblastoma. *Cancer Res.*, **69**, 4252–4259.
50. Toulany,M., Kehlbach,R., Florczak,U., Sak,A., Wang,S., Chen,J., Lobrich,M. and Rodemann,H.P. (2008) Targeting of AKT1 enhances radiation toxicity of human tumor cells by inhibiting DNA-PKcs-dependent DNA double-strand break repair. *Mol. Cancer Ther.*, **7**, 1772–1781.
51. Friedmann,B., Caplin,M., Hartley,J.A. and Hochhauser,D. (2004) Modulation of DNA repair in vitro after treatment with chemotherapeutic agents by the epidermal growth factor receptor inhibitor gefitinib (ZD1839). *Clin. Cancer Res.*, **10**, 6476–6486.
52. Bozulic,L., Surucu,B., Hynx,D. and Hemmings,B.A. (2008) PKBalpha/Akt1 acts downstream of DNA-PK in the DNA double-strand break response and promotes survival. *Mol. Cell*, **30**, 203–213.
53. Boehme,K.A., Kulikov,R. and Blattner,C. (2008) p53 stabilization in response to DNA damage requires Akt/PKB and DNA-PK. *Proce. Natl. Acad. Sci. U.S.A.*, **105**, 7785–7790.
54. Lees-Miller,S.P. (2008) PIKK-ing a new partner: a new role for PKB in the DNA damage response. *Cancer Cell*, **13**, 379–380.
55. Mahajan,K., Coppola,D., Challa,S., Fang,B., Chen,Y.A., Zhu,W., Lopez,A.S., Koomen,J., Engelman,R.W., Rivera,C. *et al.* (2010) Ack1 mediated AKT/PKB tyrosine 176 phosphorylation regulates its activation. *PLoS One*, **5**, e9646.
56. Mahajan,K., Coppola,D., Rawal,B., Chen,Y.A., Lawrence,H.R., Engelman,R.W., Lawrence,N.J. and Mahajan,N.P. (2012) Ack1-mediated androgen receptor phosphorylation modulates radiation resistance in castration-resistant prostate cancer. *J. Biol. Chem.*, **287**, 22112–22122.
57. Chou,R.H., Wang,Y.N., Hsieh,Y.H., Li,L.Y., Xia,W., Chang,W.C., Chang,L.C., Cheng,C.C., Lai,C.C., Hsu,J.L. *et al.* (2014) EGFR modulates DNA synthesis and repair through Tyr phosphorylation of histone H4. *Dev. Cell*, **30**, 224–237.
58. Sanders,S.L., Portoso,M., Mata,J., Bahler,J., Allshire,R.C. and Kouzarides,T. (2004) Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. *Cell*, **119**, 603–614.
59. Pei,H., Zhang,L., Luo,K., Qin,Y., Chesi,M., Fei,F., Bergsagel,P.L., Wang,L., You,Z. and Lou,Z. (2011) MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. *Nature*, **470**, 124–128.
60. Wang,S.C., Nakajima,Y., Yu,Y.L., Xia,W., Chen,C.T., Yang,C.C., McIntush,E.W., Li,L.Y., Hawke,D.H., Kobayashi,R. *et al.* (2006) Tyrosine phosphorylation controls PCNA function through protein stability. *Nat. Cell Biol.*, **8**, 1359–1368.
61. Ortega,J., Li,J.Y., Lee,S., Tong,D., Gu,L. and Li,G.M. (2015) Phosphorylation of PCNA by EGFR inhibits mismatch repair and promotes misincorporation during DNA synthesis. *Proc. Natl. Acad. Sci. U.S.A.*, **112**, 5667–5672.
62. Zhao,H., Lo,Y.H., Ma,L., Waltz,S.E., Gray,J.K., Hung,M.C. and Wang,S.C. (2011) Targeting tyrosine phosphorylation of PCNA inhibits prostate cancer growth. *Mol. Cancer Ther.*, **10**, 29–36.
63. Kharbanda,S., Saleem,A., Yuan,Z.M., Kraeft,S., Weichselbaum,R., Chen,L.B. and Kufe,D. (1996) Nuclear signaling induced by ionizing radiation involves colocalization of the activated p56/p53lyn tyrosine kinase with p34cdc2. *Cancer Res.*, **56**, 3617–3621.
64. Srinivasan,D. and Plattner,R. (2006) Activation of Abl tyrosine kinases promotes invasion of aggressive breast cancer cells. *Cancer Res.*, **66**, 5648–5655.
65. Tang,J., Wang,J.Y. and Parker,L.L. (2012) Detection of early Abl kinase activation after ionizing radiation by using a peptide biosensor. *Chembiochem*, **13**, 665–673.
66. Meltser,V., Ben-Yehoyada,M. and Shaul,Y. (2011) c-Abl tyrosine kinase in the DNA damage response: cell death and more. *Cell Death Differ.*, **18**, 2–4.
67. Shaul,Y. (2000) c-Abl: activation and nuclear targets. *Cell Death Differ.*, **7**, 10–16.
68. Zuckerman,V., Lenos,K., Popowicz,G.M., Silberman,I., Grossman,T., Marine,J.C., Holak,T.A., Jochemsen,A.G. and

Haupt,Y. (2009) c-Abl phosphorylates Hdmx and regulates its interaction with p53. *J. Biol. Chem.*, **284**, 4031–4039.
69. Yuan,Z.M., Shioya,H., Ishiko,T., Sun,X., Gu,J., Huang,Y.Y., Lu,H., Kharbanda,S., Weichselbaum,R. and Kufe,D. (1999) p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. *Nature*, **399**, 814–817.
70. Tsai,K.K. and Yuan,Z.M. (2003) c-Abl stabilizes p73 by a phosphorylation-augmented interaction. *Cancer Res.*, **63**, 3418–3424.
71. Mahajan,K., Challa,S., Coppola,D., Lawrence,H., Luo,Y., Gevariya,H., Zhu,W., Chen,Y.A., Lawrence,N.J. and Mahajan,N.P. (2010) Effect of Ack1 tyrosine kinase inhibitor on ligand-independent androgen receptor activity. *Prostate*, **70**, 1274–1285.
72. Mahajan,N.P., Liu,Y., Majumder,S., Warren,M.R., Parker,C.E., Mohler,J.L., Earp,H.S. and Whang,Y.E. (2007) Activated Cdc42-associated kinase Ack1 promotes prostate cancer progression via androgen receptor tyrosine phosphorylation. *Proc. Natl. Acadeedings . Sci. U.S.A.*, **104**, 8438–8443.
73. Bakkenist,C.J. and Kastan,M.B. (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature*, **421**, 499–506.
74. Li,Z., Hosoi,Y., Cai,K., Tanno,Y., Matsumoto,Y., Enomoto,A., Morita,A., Nakagawa,K. and Miyagawa,K. (2006) Src tyrosine kinase inhibitor PP2 suppresses ERK1/2 activation and epidermal growth factor receptor transactivation by X-irradiation. *Biochem. Biophys. Res. Commun.*, **341**, 363–368.
75. Kharbanda,S., Yuan,Z.M., Rubin,E., Weichselbaum,R. and Kufe,D. (1994) Activation of Src-like p56/p53lyn tyrosine kinase by ionizing radiation. *J. Biol. Chem.*, **269**, 20739–20743.
76. Kharbanda,S., Yuan,Z.M., Taneja,N., Weichselbaum,R. and Kufe,D. (1994) p56/p53lyn tyrosine kinase activation in mammalian cells treated with mitomycin C. *Oncogene*, **9**, 3005–3011.
77. Yoshida,K., Miki,Y. and Kufe,D. (2002) Activation of SAPK/JNK signaling by protein kinase Cdelta in response to DNA damage. *J. Biol. Chem.*, **277**, 48372–48378.
78. Kumar,S., Pandey,P., Bharti,A., Jin,S., Weichselbaum,R., Weaver,D., Kufe,D. and Kharbanda,S. (1998) Regulation of DNA-dependent protein kinase by the Lyn tyrosine kinase. *J. Biol. Chem.*, **273**, 25654–25658.
79. Aoyama,K., Fukumoto,Y., Ishibashi,K., Kubota,S., Morinaga,T., Horiike,Y., Yuki,R., Takahashi,A., Nakayama,Y. and Yamaguchi,N. (2011) Nuclear c-Abl-mediated tyrosine phosphorylation induces chromatin structural changes through histone modifications that include H4K16 hypoacetylation. *Exp. Cell Res.*, **317**, 2874–2903.
80. Keshet,R., Adler,J., Ricardo Lax,I., Shanzer,M., Porat,Z., Reuven,N. and Shaul,Y. (2015) c-Abl antagonizes the YAP oncogenic function. *Cell Death Differ.*, **22**, 935–945.
81. Hartwell,L.H., Culotti,J., Pringle,J.R. and Reid,B.J. (1974) Genetic control of the cell division cycle in yeast. *Science (New York, N.Y.)*, **183**, 46–51.
82. Mahajan,K. and Mahajan,N.P. (2013) WEE1 tyrosine kinase, a novel epigenetic modifier. *Trends Genet.*, **29**, 394–402.
83. Meeks-Wagner,D., Wood,J.S., Garvik,B. and Hartwell,L.H. (1986) Isolation of two genes that affect mitotic chromosome transmission in S. cerevisiae. *Cell*, **44**, 53–63.
84. Wu,G., Broniscer,A., McEachron,T.A., Lu,C., Paugh,B.S., Becksfort,J., Qu,C., Ding,L., Huether,R., Parker,M. *et al.* (2012) Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat. Genet.*, **44**, 251–253.
85. Schwartzentruber,J., Korshunov,A., Liu,X.Y., Jones,D.T., Pfaff,E., Jacob,K., Sturm,D., Fontebasso,A.M., Quang,D.A., Tonjes,M. *et al.* (2012) Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*, **482**, 226–231.
86. Wolffe,A.P. (1996) Chromatin and gene regulation at the onset of embryonic development. *Reprod. Nutr. Dev.*, **36**, 581–606.
87. Gunjan,A. and Verreault,A. (2003) A Rad53 kinase-dependent surveillance mechanism that regulates histone protein levels in S. cerevisiae. *Cell*, **115**, 537–549.
88. Singh,R.K., Kabbaj,M.H., Paik,J. and Gunjan,A. (2009) Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. *Nat. Cell Biol.*, **11**, 925–933.
89. Singh,R.K., Liang,D., Gajjalaiahvari,U.R., Kabbaj,M.H., Paik,J. and Gunjan,A. (2010) Excess histone levels mediate cytotoxicity via multiple mechanisms. *Cell Cycle (Georgetown, Tex)*, **9**, 4236–4244.
90. Holzen,T.M. and Sclafani,R. (2010) Genetic interaction of RAD53 protein kinase with histones is important for DNA replication. *Cell Cycle (Georgetown, Tex)*, **9**, 4735–4747.
91. Chen,S.H., Albuquerque,C.P., Liang,J., Suhandynata,R.T. and Zhou,H. (2010) A proteome-wide analysis of kinase-substrate network in the DNA damage response. *J. Biol. Chem.*, **285**, 12803–12812.
92. Mahajan,K., Fang,B., Koomen,J.M. and Mahajan,N.P. (2012) H2B Tyr37 phosphorylation suppresses expression of replication-dependent core histone genes. *Nat. Struct. Mol. Biol.*, **19**, 930–937.
93. Dawson,M.A., Bannister,A.J., Gottgens,B., Foster,S.D., Bartke,T., Green,A.R. and Kouzarides,T. (2009) JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature*, **461**, 819–822.
94. Pacaud,R., Cheray,M., Nadaradjane,A., Vallette,F.M. and Cartron,P.F. (2015) Histone H3 phosphorylation in GBM: a new rational to guide the use of kinase inhibitors in anti-GBM therapy. *Theranostics*, **5**, 12–22.
95. Chitnis,M.M., Lodhia,K.A., Aleksic,T., Gao,S., Protheroe,A.S. and Macaulay,V.M. (2014) IGF-1R inhibition enhances radiosensitivity and delays double-strand break repair by both non-homologous end-joining and homologous recombination. *Oncogene*, **33**, 5262–5273.
96. Turney,B.W., Kerr,M., Chitnis,M.M., Lodhia,K., Wang,Y., Riedemann,J., Rochester,M., Protheroe,A.S., Brewster,S.F. and Macaulay,V.M. (2012) Depletion of the type 1 IGF receptor delays repair of radiation-induced DNA double strand breaks. *Radiother. Oncol.*, **103**, 402–409.
97. Yang,S., Chintapalli,J., Sodagum,L., Baskin,S., Malhotra,A., Reiss,K. and Meggs,L.G. (2005) Activated IGF-1R inhibits hyperglycemia-induced DNA damage and promotes DNA repair by homologous recombination. *Am. J. Physiol. Renal Physiol.*, **289**, F1144–F1152.
98. Slupianek,A., Hoser,G., Majsterek,I., Bronisz,A., Malecki,M., Blasiak,J., Fishel,R. and Skorski,T. (2002) Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G(2)/M phase, and protection from apoptosis. *Mol. Cell. Biol.*, **22**, 4189–4201.
99. Baskaran,R., Wood,L.D., Whitaker,L.L., Canman,C.E., Morgan,S.E., Xu,Y., Barlow,C., Baltimore,D., Wynshaw-Boris,A., Kastan,M.B. *et al.* (1997) Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. *Nature*, **387**, 516–519.
100. Kharbanda,S., Pandey,P., Jin,S., Inoue,S., Bharti,A., Yuan,Z.M., Weichselbaum,R., Weaver,D. and Kufe,D. (1997) Functional interaction between DNA-PK and c-Abl in response to DNA damage. *Nature*, **386**, 732–735.
101. Sun,Y., Jiang,X., Chen,S., Fernandes,N. and Price,B.D. (2005) A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc. Natl. Acad. Sci. U.S.A.*, **102**, 13182–13187.
102. Sun,Y., Jiang,X., Xu,Y., Ayrapetov,M.K., Moreau,L.A., Whetstine,J.R. and Price,B.D. (2009) Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. *Nat. Cell Biol.*, **11**, 1376–1382.
103. Jiang,Z., Kamath,R., Jin,S., Balasubramani,M., Pandita,T.K. and Rajasekaran,B. (2011) Tip60-mediated acetylation activates transcription independent apoptotic activity of Abl. *Mol. Cancer*, **10**, 88.
104. Kharbanda,S., Yuan,Z.M., Weichselbaum,R. and Kufe,D. (1997) Functional role for the c-Abl protein tyrosine kinase in the cellular response to genotoxic stress. *Biochim. Biophys. Acta*, **1333**, O1–O7.
105. Wang,J.Y. (2004) Controlling Abl: auto-inhibition and co-inhibition? *Nat. Cell Biol.*, **6**, 3–7.
106. Kumar,V., Sabatini,D., Pandey,P., Gingras,A.C., Majumder,P.K., Kumar,M., Yuan,Z.M., Carmichael,G., Weichselbaum,R., Sonenberg,N. *et al.* (2000) Regulation of the rapamycin and FKBP-target 1/mammalian target of rapamycin and cap-dependent initiation of translation by the c-Abl protein-tyrosine kinase. *J. Biol. Chem.*, **275**, 10779–10787.

107. Yuan,Z.M., Utsugisawa,T., Huang,Y., Ishiko,T., Nakada,S., Kharbanda,S., Weichselbaum,R. and Kufe,D. (1997) Inhibition of phosphatidylinositol 3-kinase by c-Abl in the genotoxic stress response. *J. Biol. Chem.*, **272**, 23485–23488.
108. Chen,G., Yuan,S.S., Liu,W., Xu,Y., Trujillo,K., Song,B., Cong,F., Goff,S.P., Wu,Y., Arlinghaus,R. *et al.* (1999) Radiation-induced assembly of Rad51 and Rad52 recombination complex requires ATM and c-Abl. *J. Biol. Chem.*, **274**, 12748–12752.
109. Shimizu,H., Popova,M., Fleury,F., Kobayashi,M., Hayashi,N., Sakane,I., Kurumizaka,H., Venkitaraman,A.R., Takahashi,M. and Yamamoto,K. (2009) c-ABL tyrosine kinase stabilizes RAD51 chromatin association. *Biochem. Biophys. Res. Commun.*, **382**, 286–291.
110. Kitao,H. and Yuan,Z.M. (2002) Regulation of ionizing radiation-induced Rad52 nuclear foci formation by c-Abl-mediated phosphorylation. *J. Biol. Chem.*, **277**, 48944–48948.
111. Honda,M., Okuno,Y., Yoo,J., Ha,T. and Spies,M. (2011) Tyrosine phosphorylation enhances RAD52-mediated annealing by modulating its DNA binding. *EMBO J.*, **30**, 3368–3382.
112. Xiao,A., Li,H., Shechter,D., Ahn,S.H., Fabrizio,L.A., Erdjument-Bromage,H., Ishibe-Murakami,S., Wang,B., Tempst,P., Hofmann,K. *et al.* (2009) WSTF regulates the H2A.*X DNA damage response via a novel tyrosine kinase activity*. *Nature*, **457**, 57–62.
113. Cook,P.J., Ju,B.G., Telese,F., Wang,X., Glass,C.K. and Rosenfeld,M.G. (2009) Tyrosine dephosphorylation of H2AX modulates apoptosis and survival decisions. *Nature*, **458**, 591–596.
114. Singh,N., Basnet,H., Wiltshire,T.D., Mohammad,D.H., Thompson,J.R., Heroux,A., Botuyan,M.V., Yaffe,M.B., Couch,F.J., Rosenfeld,M.G. *et al.* (2012) Dual recognition of phosphoserine and phosphotyrosine in histone variant H2A.X by DNA damage response protein MCPH1. *Proc. Nat. Acad. Sci. U.S.A.*, **109**, 14381–14386.
115. Brown,J.A., Eykelenboom,J.K. and Lowndes,N.F. (2012) Co-mutation of histone H2AX S139A with Y142A rescues Y142A-induced ionising radiation sensitivity. *FEBS Open Bio*, **2**, 313–317.
116. Sone,K., Piao,L., Nakakido,M., Ueda,K., Jenuwein,T., Nakamura,Y. and Hamamoto,R. (2014) Critical role of lysine 134 methylation on histone H2AX for gamma-H2AX production and DNA repair. *Nat. Commun.*, **5**, 5691.
117. Mahajan,K.N. and Mitchell,B.S. (2003) Role of human Pso4 in mammalian DNA repair and association with terminal deoxynucleotidyl transferase. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 10746–10751.
118. Dellago,H., Khan,A., Nussbacher,M., Gstraunthaler,A., Lammermann,I., Schosserer,M., Muck,C., Anrather,D., Scheffold,A., Ammerer,G. *et al.* (2012) ATM-dependent phosphorylation of SNEVhPrp19/hPso4 is involved in extending cellular life span and suppression of apoptosis. *Aging*, **4**, 290–304.
119. Beck,B.D., Park,S.J., Lee,Y.J., Roman,Y., Hromas,R.A. and Lee,S.H. (2008) Human Pso4 is a metnase (SETMAR)-binding partner that regulates metnase function in DNA repair. *J. Biol. Chem.*, **283**, 9023–9030.
120. Beck,B.D., Lee,S.S., Hromas,R. and Lee,S.H. (2010) Regulation of Metnase's TIR binding activity by its binding partner, Pso4. *Arch. Biochem. Biophys.*, **498**, 89–94.
121. Kubota,S., Morii,M., Yuki,R., Yamaguchi,N., Yamaguchi,H., Aoyama,K., Kuga,T., Tomonaga,T. and Yamaguchi,N. (2015) Role for Tyrosine Phosphorylation of A-kinase Anchoring Protein 8 (AKAP8) in Its Dissociation from Chromatin and the Nuclear Matrix. *J. Biol. Chem.*, **290**, 10891–10904.
122. Castello,A., Fischer,B., Eichelbaum,K., Horos,R., Beckmann,B.M., Strein,C., Davey,N.E., Humphreys,D.T., Preiss,T., Steinmetz,L.M. *et al.* (2012) Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell*, **149**, 1393–1406.
123. Eide,T., Coghlan,V., Orstavik,S., Holsve,C., Solberg,R., Skalhegg,B.S., Lamb,N.J., Langeberg,L., Fernandez,A., Scott,J.D. *et al.* (1998) Molecular cloning, chromosomal localization, and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95. *Exp. Cell Res.*, **238**, 305–316.
124. Guo,Z., Dai,B., Jiang,T., Xu,K., Xie,Y., Kim,O., Nesheiwat,I., Kong,X., Melamed,J., Handratta,V.D. *et al.* (2006) Regulation of androgen receptor activity by tyrosine phosphorylation. *Cancer Cell*, **10**, 309–319.
125. Lim,D.S. and Hasty,P. (1996) A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol. Cell. Biol.*, **16**, 7133–7143.
126. Tsuzuki,T., Fujii,Y., Sakumi,K., Tominaga,Y., Nakao,K., Sekiguchi,M., Matsushiro,A., Yoshimura,Y. and Morita,T. (1996) Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 6236–6240.
127. Fortschegger,K., Wagner,B., Voglauer,R., Katinger,H., Sibilia,M. and Grillari,J. (2007) Early embryonic lethality of mice lacking the essential protein SNEV. *Mol. Cell. Biol.*, **27**, 3123–3130.
128. Tominaga,Y., Li,C., Wang,R.H. and Deng,C.X. (2006) Murine Wee1 plays a critical role in cell cycle regulation and pre-implantation stages of embryonic development. *Int. J. Biol. Sci.*, **2**, 161–170.
129. Bhosle,J., Kiakos,K., Porter,A.C., Wu,J., Makris,A., Hartley,J.A. and Hochhauser,D. (2013) Treatment with gefitinib or lapatinib induces drug resistance through downregulation of topoisomerase IIalpha expression. *Mol. Cancer Ther.*, **12**, 2897–2908.
130. Kim,J.C., Ali,M.A., Nandi,A., Mukhopadhyay,P., Choy,H., Cao,C. and Saha,D. (2005) Correlation of HER1/EGFR expression and degree of radiosensitizing effect of the HER1/EGFR-tyrosine kinase inhibitor erlotinib. *Indian J. Biochem. Biophys.*, **42**, 358–365.
131. Raju,U., Riesterer,O., Wang,Z.Q., Molkentine,D.P., Molkentine,J.M., Johnson,F.M., Glisson,B., Milas,L. and Ang,K.K. (2012) Dasatinib, a multi-kinase inhibitor increased radiation sensitivity by interfering with nuclear localization of epidermal growth factor receptor and by blocking DNA repair pathways. *Radiother. Oncol.*, **105**, 241–249.
132. Sambade,M.J., Camp,J.T., Kimple,R.J., Sartor,C.I. and Shields,J.M. (2009) Mechanism of lapatinib-mediated radiosensitization of breast cancer cells is primarily by inhibition of the Raf>MEK>ERK mitogen-activated protein kinase cascade and radiosensitization of lapatinib-resistant cells restored by direct inhibition of MEK. *Radiother. Oncol.*, **93**, 639–644.
133. Dittmann,K., Mayer,C., Fehrenbacher,B., Schaller,M., Raju,U., Milas,L., Chen,D.J., Kehlbach,R. and Rodemann,H.P. (2005) Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. *J. Biol. Chem.*, **280**, 31182–31189.
134. Liccardi,G., Hartley,J.A. and Hochhauser,D. (2011) EGFR nuclear translocation modulates DNA repair following cisplatin and ionizing radiation treatment. *Cancer Res.*, **71**, 1103–1114.
135. Baro,M., de Llobet,L.I., Figueras,A., Skvortsova,I., Mesia,R. and Balart,J. (2014) Dasatinib worsens the effect of cetuximab in combination with fractionated radiotherapy in FaDu- and A431-derived xenografted tumours. *Br. J. Cancer*, **111**, 1310–1318.
136. Majsterek,I., Blasiak,J., Mlynarski,W., Hoser,G. and Skorski,T. (2002) Does the bcr/abl-mediated increase in the efficacy of DNA repair play a role in the drug resistance of cancer cells? *Cell Biol. Int.*, **26**, 363–370.
137. Tobin,L.A., Robert,C., Rapoport,A.P., Gojo,I., Baer,M.R., Tomkinson,A.E. and Rassool,F.V. (2013) Targeting abnormal DNA double-strand break repair in tyrosine kinase inhibitor-resistant chronic myeloid leukemias. *Oncogene*, **32**, 1784–1793.
138. Canitrot,Y., Falinski,R., Louat,T., Laurent,G., Cazaux,C., Hoffmann,J.S., Lautier,D. and Skorski,T. (2003) p210 BCR/ABL kinase regulates nucleotide excision repair (NER) and resistance to UV radiation. *Blood*, **102**, 2632–2637.
139. Qiao,B., Kerr,M., Groselj,B., Teo,M.T., Knowles,M.A., Bristow,R.G., Phillips,R.M. and Kiltie,A.E. (2013) Imatinib radiosensitizes bladder cancer by targeting homologous recombination. *Cancer Res.*, **73**, 1611–1620.
140. Yadav,A., Kumar,B., Teknos,T.N. and Kumar,P. (2011) Sorafenib enhances the antitumor effects of chemoradiation treatment by downregulating ERCC-1 and XRCC-1 DNA repair proteins. *Mol. Cancer Ther.*, **10**, 1241–1251.
141. Mahajan,K. and Mahajan,N.P. (2014) ACK1/TNK2 tyrosine kinase: molecular signaling and evolving role in cancers. *Oncogene*.
142. Mahajan,K., Lawrence,H.R., Lawrence,N.J. and Mahajan,N.P. (2014) ACK1 tyrosine kinase interacts with histone demethylase KDM3A to regulate the mammary tumor oncogene HOXA1. *J. Biol. Chem.*, **289**, 28179–28191.

143. Okada,Y., Scott,G., Ray,M.K., Mishina,Y. and Zhang,Y. (2007) Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis. *Nature*, **450**, 119–123.
144. Yamane,K., Toumazou,C., Tsukada,Y., Erdjument-Bromage,H., Tempst,P., Wong,J. and Zhang,Y. (2006) JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell*, **125**, 483–495.
145. Mir,S.E., De Witt Hamer,P.C., Krawczyk,P.M., Balaj,L., Claes,A., Niers,J.M., Van Tilborg,A.A., Zwinderman,A.H., Geerts,D., Kaspers,G.J. *et al.* (2010) In silico analysis of kinase expression identifies WEE1 as a gatekeeper against mitotic catastrophe in glioblastoma. *Cancer Cell*, **18**, 244–257.
146. De Witt Hamer,P.C., Mir,S.E., Noske,D., Van Noorden,C.J. and Wurdinger,T. (2011) WEE1 kinase targeting combined with DNA-damaging cancer therapy catalyzes mitotic catastrophe. *Clini. Cancer Res.*, **17**, 4200–4207.
147. Hirai,H., Arai,T., Okada,M., Nishibata,T., Kobayashi,M., Sakai,N., Imagaki,K., Ohtani,J., Sakai,T., Yoshizumi,T. *et al.* (2010) MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. *Cancer Biol. Ther.*, **9**, 514–522.
148. Hong,M.K., Macintyre,G., Wedge,D.C., Van Loo,P., Patel,K., Lunke,S., Alexandrov,L.B., Sloggett,C., Cmero,M., Marass,F. *et al.* (2015) Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. *Nat. Commun.*, **6**, 6605.
149. Goodwin,J.F., Schiewer,M.J., Dean,J.L., Schrecengost,R.S., de Leeuw,R., Han,S., Ma,T., Den,R.B., Dicker,A.P., Feng,F.Y. *et al.* (2013) A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discovery*, **3**, 1254–1271.
150. Goldberg,Z., Vogt Sionov,R., Berger,M., Zwang,Y., Perets,R., Van Etten,R.A., Oren,M., Taya,Y. and Haupt,Y. (2002) Tyrosine phosphorylation of Mdm2 by c-Abl: implications for p53 regulation. *EMBO J.*, **21**, 3715–3727.
151. Dias,S.S., Milne,D.M. and Meek,D.W. (2006) c-Abl phosphorylates Hdm2 at tyrosine 276 in response to DNA damage and regulates interaction with ARF. *Oncogene*, **25**, 6666–6671.
152. Chen,X., Gohain,N., Zhan,C., Lu,W.Y., Pazgier,M. and Lu,W. (2015) Structural basis of how stress-induced MDMX phosphorylation activates p53. *Oncogene*.
153. Agami,R., Blandino,G., Oren,M. and Shaul,Y. (1999) Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. *Nature*, **399**, 809–813.
154. Gonfloni,S., Di Tella,L., Caldarola,S., Cannata,S.M., Klinger,F.G., Di Bartolomeo,C., Mattei,M., Candi,E., De Felici,M., Melino,G. *et al.* (2009) Inhibition of the c-Abl-TAp63 pathway protects mouse oocytes from chemotherapy-induced death. *Nat. Med.*, **15**, 1179–1185.
155. Yi,W., Lee,T.H., Tompkins,J.D., Zhu,F., Wu,X. and Her,C. (2006) Physical and functional interaction between hMSH5 and c-Abl. *Cancer Res.*, **66**, 151–158.
156. Jin,S., Kharbanda,S., Mayer,B., Kufe,D. and Weaver,D.T. (1997) Binding of Ku and c-Abl at the kinase homology region of DNA-dependent protein kinase catalytic subunit. *J. Biol. Chem.*, **272**, 24763–24766.
157. Foray,N., Marot,D., Randrianarison,V., Venezia,N.D., Picard,D., Perricaudet,M., Favaudon,V. and Jeggo,P. (2002) Constitutive association of BRCA1 and c-Abl and its ATM-dependent disruption after irradiation. *Mol. Cell. Biol.*, **22**, 4020–4032.
158. Lee,H.J., Lan,L., Peng,G., Chang,W.C., Hsu,M.C., Wang,Y.N., Cheng,C.C., Wei,L., Nakajima,S., Chang,S.S. *et al.* (2015) Tyrosine 370 phosphorylation of ATM positively regulates DNA damage response. *Cell Res.*, **25**, 225–236.
159. Russell,P. and Nurse,P. (1987) Negative regulation of mitosis by wee1+, a gene encoding a protein kinase homolog. *Cell*, **49**, 559–567.
160. Rhind,N., Furnari,B. and Russell,P. (1997) Cdc2 tyrosine phosphorylation is required for the DNA damage checkpoint in fission yeast. *Genes Dev.*, **11**, 504–511.

Oncogene (2015), 1–8

www.nature.com/onc

REVIEW

hPso4/hPrp19: a critical component of DNA repair and DNA damage checkpoint complexes

K Mahajan

Genome integrity is vital to cellular homeostasis and its forfeiture is linked to deleterious consequences—cancer, immunodeficiency, genetic disorders and premature aging. The human ubiquitin ligase Pso4/Prp19 has emerged as a critical component of multiple DNA damage response (DDR) signaling networks. It not only senses DNA damage, binds double-stranded DNA in a sequence-independent manner, facilitates processing of damaged DNA, promotes DNA end joining, regulates replication protein A (RPA2) phosphorylation and ubiquitination at damaged DNA, but also regulates RNA splicing and mitotic spindle formation in its integral capacity as a scaffold for a multimeric core complex. Accordingly, by virtue of its regulatory and structural interactions with key proteins critical for genome integrity—DNA double-strand break (DSB) repair, DNA interstrand crosslink repair, repair of stalled replication forks and DNA end joining—it fills a unique niche in restoring genomic integrity after multiple types of DNA damage and thus has a vital role in maintaining chromatin integrity and cellular functions. These properties may underlie its ability to thwart replicative senescence and, not surprisingly, have been linked to the self-renewal and colony-forming ability of murine hematopoietic stem cells. This review highlights recent advances in hPso4 research that provides a fascinating glimpse into the pleiotropic activities of a ubiquitously expressed multifunctional E3 ubiquitin ligase.

Oncogene advance online publication, 14 September 2015; doi:10.1038/onc.2015.321

INTRODUCTION

A hierarchy of molecular events is initiated following DNA damage via the activation of the DNA damage response (DDR) networks that expedite DNA repair, promote genetic integrity, ensure genetic stability and avert replicative senescence.[1,2] Failure to restore genome integrity also potentiates pathological outcomes—chromosomal translocations, fragmented chromosomes, missegregation of chromosomes and aneuploidy—and is linked to hereditary disorders, cancer as well as premature aging.[3,4] Accordingly, genome integrity depends upon all aspects of DNA metabolic activities—DNA replication, recombination, chromosome segregation and epigenetic inheritance. Depending on the type of DNA damage multiple protein networks are activated and thus undertake critical roles as tumor suppressors.[5–7] Not surprisingly, factors important for maintaining genomic integrity are evolutionarily conserved, retaining similar functionality, catalytic activities and interacting partners at damaged sites.[8,9] This highly orchestrated strategy developed by unicellular to multicellular organisms is necessary to combat both endogenous and exogenous agents targeting the integrity of the genome (Figure 1). A number of the DNA double-strand break (DSB) repair proteins, particularly members of the nonhomologous end joining (NHEJ) pathway, have critical roles in V(D)J recombination—a process employed to generate immunoglobulin and T cell receptor diversity during lymphocyte development. Hence, defects in these genes are also linked to immunodeficiency.[10–13]

DNA damage can occur in all phases of the cell cycle: during G1 (pre-DNA synthesis), S (DNA synthesis), G2 (post-DNA synthesis/pre-mitosis) and M (mitosis with chromosome segregation and cytokinesis) phases. DNA damage can be inflicted by exogenous agents such as ultraviolet radiation (UV), ionizing radiation (IR), radiomimetic compounds, chemical carcinogens and endogenous agents such as reactive oxygen species and endonucleases (Figure 1). DNA damage can take the form of interstrand crosslinks (ICLs): single-strand breaks and DSBs. To all these insidious attacks, the mammalian cells respond by activating the DNA damage checkpoint pathways that initiate signaling cascades to rapidly arrest the cells at a specific stage in the cell cycle as well as recruiting damage-specific repair (homologous recombination, NHEJ, Alt-NHEJ, ICL repair) complexes such as the NHEJ throughout the cell cycle and homologous recombination during the late S and G2 phases.[14] Under these exigent circumstances, multiprotein complexes coordinately act at the damage sites in a well-orchestrated manner to restore genomic integrity.[15–18] Defects in the DNA repair proteins and DDR pathways is also linked to premature aging, cancer predisposition and neurodegenerative disorders.[19–21] Although it may appear that these are disparate pathways, some proteins work across the defined distinctions to facilitate DNA repair. hPso4/Prp19, the subject of this article, is an example of a protein with pleiotropic activities in DNA damage signaling, repair and replicative senescence.

Pso4 STRUCTURE–FUNCTION ANALYSIS

Genetic studies in the budding yeast *Saccharomyces cerevisiae* have laid the foundation for a number of genes important for preserving genome stability.[22–24] The *ScPSO4* gene, first identified as a *pso*ralen-sensitive mutant 4 (*pso4-1*) for its inability to repair photoactivated 8 methoxy psoralen-induced DNA ICLs, was found

Tumor Biology Department and Department of Oncological Sciences, Moffitt Cancer Center, University of South Florida, Tampa, FL, USA. Correspondence: Dr K Mahajan, Tumor Biology Department and Department of Oncological Sciences, Moffitt Cancer Center, University of South Florida, 12902, Magnolia Drive, Tampa, FL 33612, USA.
E-mail: kiran.mahajan@moffitt.org
Received 27 May 2015; revised 16 July 2015; accepted 19 July 2015

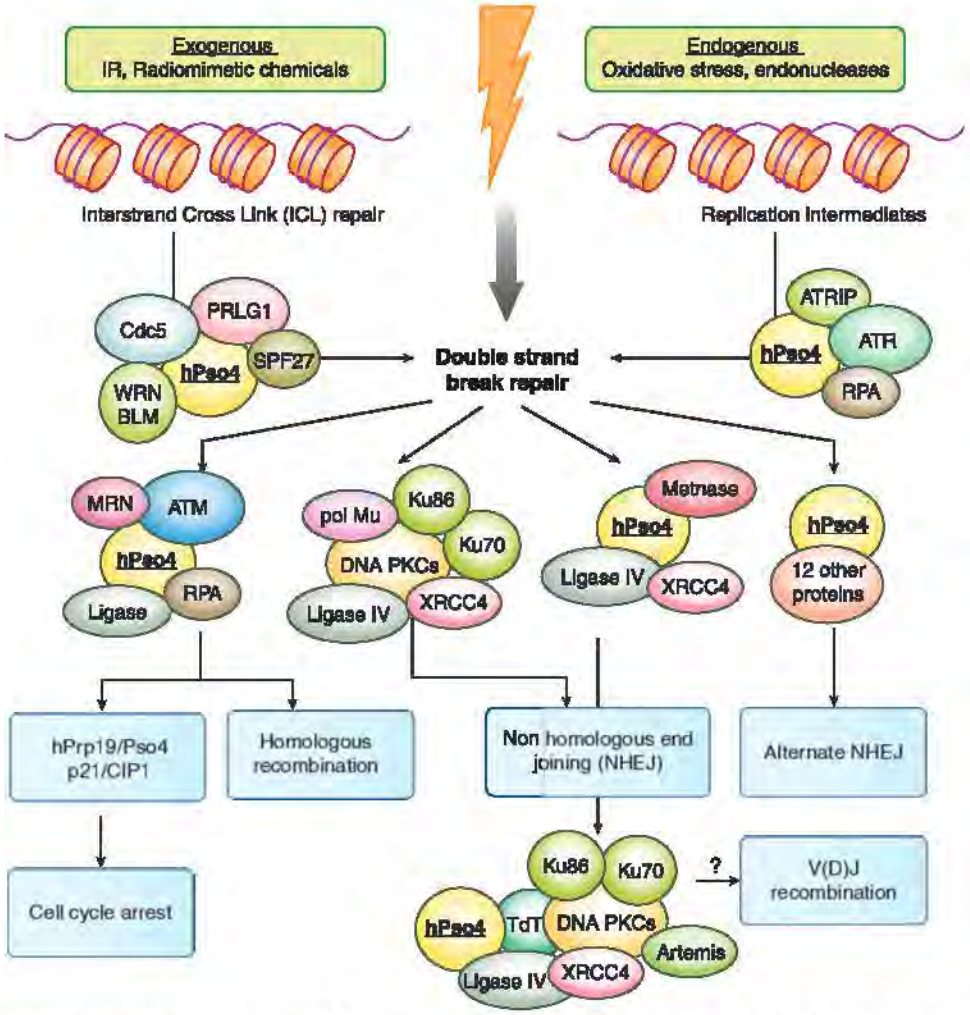


Figure 1. hPso4/hPrp19, an integral component of DDR machinery and essential for genome integrity. hPso4 operates across multiple DNA damage response pathways by dynamically associating with various protein complexes. It interacts with chromatin, recruits DNA repair proteins following DNA damage and is essential for recognition, processing and repair of lesions caused by a variety of DNA-damaging agents. The hPso4 core complex includes Cdc5L, Spf27 and Plrg1 and functions in the processing of the ICLs. hPso4–Cdc5L complex may stabilize the WRN helicase at ICLs via the interaction of Cdc5L with WRN. hPso4/Prp19 also functions at stalled replication forks and is required to activate the ATR/Chk1-dependent replication stress checkpoint pathways. It colocalizes with proliferating cell nuclear antigen (PCNA), interacts with the RPA and is required for replication fork stability or restart after hydroxyurea-induced DNA damage. hPso4 is modified at S149 by ATM kinase. Formation of DSB induced Nbs1 foci are impaired in hPso4 deficient cells. hPso4 maintains genome stability by regulating homologous recombination. Human Pso4 was initially identified as a specific interactor of the BRCT (Breast Cancer Carboxy terminal, BRCA1) domain of the template-independent DNA polymerase. TdT also interacts with DNAPK complex and catalyzes random N nucleotide addition during V(D)J recombination. Pso4 is also required for Alt-NHEJ (alternative end joining) or MMHEJ (micro homology-mediated end joining) in a Ku-independent manner. hPso4 functions as a DNA binding partner for a transposase-like protein called Metnase/SETMAR, may facilitate binding of this heteromeric complex to non-terminal inverted repeat (TIR) DNA sequences and is essential for the recruitment of Metnase to DSBs. Prp19/ Pso4 upregulation promotes cell cycle arrest by upregulating p21 levels.

to be essential in haploid cells and allelic to *prp19*, a gene essential for mRNA splicing.[25,26] Its ortholog in humans, the PSO4/PRP19F, has been independently uncovered by different laboratories as a constituent of the nuclear matrix (hNMP200),[27] DNA repair protein (hPso4),[28] senescence-associated protein (senescence evasion factor (SNEV))[29] and mRNA splicing apparatus (Prp19).[30] Although the human and yeast hPso4/Prp19 display only a 23% identity and 41% similarity in amino acid composition, remarkably, they display a high degree of functional conservation and domain architecture.[28,29] Pso4/Prp19 is a 55-kDa protein (Figure 2) and is composed of an N-terminal U box—a ubiquitin ligase domain with E2 recruitment function that facilitates dimerization[31–33] and is essential for its auto-ubiquitination activity *in vitro* or when overexpressed.[33–35] Importantly, the L15E mutation in the U box of the yeast Prp19 disrupts U-box dimerization and compromises cell viability.[33] The central coiled-coil Prp19 homology region mediates oligomerization (tetramer) of the protein.[35] The carboxy terminus has a seven-bladed WD40 β-propeller type of leucine-rich architectural repeats that form an asymmetrical barrel-shaped structure that is important for substrate recognition and recruitment.[31–33,35–37] Sequence analysis of the ScPso4/Prp19 revealed a myb-like DNA binding domain at the C-terminus (positions 457–465, WTKDEESAL),[26] consistent with its role as a DNA binding protein.[28] Recent studies aimed at understanding the role of hPrp19/Pso4 in splicing provide a deeper understanding of how its E3 ubiquitin ligase activity embedded in the U box, coupled with its scaffolding function via the WD40 repeats, regulates the assembly of multiprotein complexes.[38] It is an essential member of a core complex of four proteins (Pso4/Prp19, Cdc5, PLRG1 and BCAS2/SPF27) that are required before the

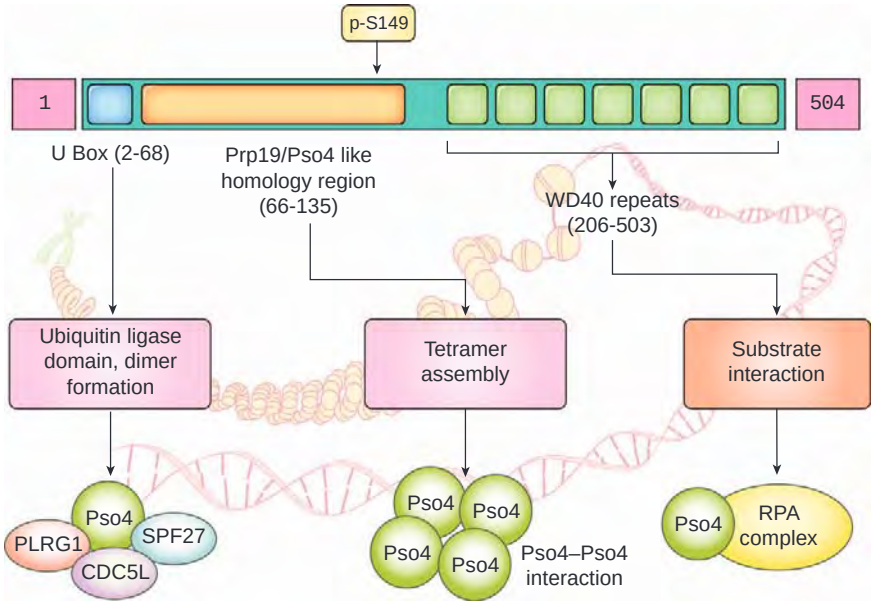


Figure 2. Structure–function studies of Pso4. The human and yeast hPso4/Prp19 display a high degree of functional conservation and domain architecture. Pso4/Prp19 is a 55-kDa protein and is composed of an N-terminal U box—a ubiquitin ligase domain with E2 recruitment function that facilitates dimerization and is essential for its auto-ubiquitination activity. It has a central coiled-coil Prp19 homology region that mediates oligomerization (tetramer) of the protein, and seven-bladed WD40 β-propeller type of architectural repeats that forms an asymmetrical barrel-shaped structure and functions as a scaffold for substrate recognition and recruitment. hPso4 is phosphorylated at S149 in an ATM-dependent manner in response to oxidative stress.

Table 1. Pso4/SNEV/Prp19 interacting proteins

Protein	*Function*	*Molecular process*	*References*
Terminal deoxynucleotidyl transferase	Template-independent DNA polymerase	VDJ recombination	28
hCdc5L, PLRG1, SPF27/BCAS2	Splicing factor	Pre-mRNA splicing, DNA damage signaling, mitotic spindle assembly	28,87,92,93
WRN protein (via Cdc5L)	DNA helicase	Interstrand crosslink repair	46
PSMB4	β7 subunit of the 20S proteasome	Ubiquitin–proteasome system for protein degradation	94
Blom7α	Splicing factor	Pre-mRNA splicing	93
Metnase (also known as SETMAR)	SET and transposase fusion protein	Double-strand break (DSB) repair	47
Exo70	Exocyst complex	Pre-mRNA splicing	95
Replication protein A	Single-stranded DNA binding protein	Replication/repair	62,63
Ataxia telangiectasia-mutated and Rad3 related (ATR)/ATRIP	Regulator of DNA damage signaling by stalled replication forks	DNA damage signaling	62,63
Ataxia telangiectasia-mutated (ATM)	Regulator of DNA damage signaling by double-strand breaks	DNA damage signaling	4

first step of splicing.[39] Together, they regulate mRNA processing by imparting stability to the U4/U5/U6 snRNP spliceosomal complex.[30,37,40–42] Pso4 interacts with the U4 spliceosomal protein Prp3 via its WD40 repeat region and promotes multi-ubiquitination of the C terminal domain of Prp3 using the lysine 63-linked ubiquitin chains (K63). This post-translational modification appears to stabilize the U4/U5/U6 snRNP spliceosomal complex by promoting interaction of the ubiquitinated Prp3 with U5 spliceosomal protein Prp8 and is not a signal for proteolytic degradation.[38] Furthermore, in conjunction with another splicing factor, U2AF65, that interacts with the large subunit of RNAPII, hPso4 complex forms a physical link in coupling transcription with RNA splicing.[42] In contrast to its role in modifying other proteins during RNA splicing, auto-ubiquitylation of hPso4 and ubiquitylation of replication protein A (RPA) is reported following DNA damage.[43,44] Moreover, ubiquitylation particularly after DNA damage appears to be important for its dissociation from two of the core complex proteins Cdc5L and PLRG1, providing clues to how the self-directed modifications of Pso4 may underlie its ability to switch between different macromolecular entities (Table 1). PSO4 assembles as oligomers following DNA damage and requires the U box, but not its E3 ubiquitin ligase activity for this self-association, suggesting another interesting aspect of its functionality.[37,45] Whether ubiquitylated Pso4 is recognized by specific DDR factors for their recruitment at damage sites is not known. Nevertheless, structure–function studies provide insights into how different domains in Pso4 regulate intramolecular and intermolecular interactions to promote its different functions. It fundamentally may employ a similar modus operandi to regulate proper RNA processing and DNA processing events by utilizing its domains effectively to communicate with complex cellular machineries in all eukaryotes.

hPso4 IN DNA DSB REPAIR

An important role for hPso4 is its requirement in multiple DDR pathways (Figure 1). hPso4 protein is induced in cells by a variety of DSB-inducing agents such as γ-radiation, cisplatin, mitomycin C and bleomycin, and it is essential for recognition, processing and repair of DNA lesions.[28,46,47] Conversely, overexpression of

hPso4 exerts a protective effect against DNA damage-induced apoptosis.[43] Indeed, the *Scpso4/prp19* exhibits sensitivity to a variety of genotoxic agents such as X-rays and photoactivated 8 methoxy psoralen. It appears to functionally participate with either the members of the error prone translesion DNA repair pathways or the homologous recombinational repair pathways to repair the specific DNA damage and has therefore been assigned to different epistasis groups.[26,48,49] Mammalian cells with loss of PSO4 expression by small interfering RNA interference displayed exquisite sensitivity to DSB-forming agents such as IR and etoposide, and the ICL-generating agent, mitomycin C, that could be attributed to their inability to repair DSBs.[28] At the molecular level, hPso4 binds double-stranded DNA,[28,47] and its interaction with chromatin is enhanced following mitomycin C-induced DNA damage.[43] In Pso4 knockdown cells the IR-induced Nbs1 foci was impaired suggesting a poorly understood role for Pso4 in the recruitment or stabilization of proteins involved in the early steps of DNA damage signaling.[47] Taken together, these data indicate that PSO4 is a part of the DDR machinery that is recruited specifically to the sites of DNA damage.

hPso4 INTERACTION WITH DNA DAMAGE CHECKPOINT KINASES

At the helm of the DNA damage response pathway are three variant but related members of phosphotidylinositol 3-kinase (PI3K) family that includes ATM (mutated in ataxia telangiectasia), ATR (ATM and Rad3 related) and DNA-PK (DNA-dependent protein kinase) that function as serine/threonine protein kinases.[50] Of these, the 370 kDa nuclear phosphoprotein ATM appears to be pivotal for coordinating multifaceted cellular responses to DSBs. Biallelic mutations in the ATM gene in human patients have pleiotropic effects—impaired cell cycle checkpoint activation, DNA repair defects, defective response to growth stimuli that are discernible as radiation hypersensitivity and genome instability, predisposition to lymphoid malignancies, abnormalities of neurological and vascular type, cerebellar degeneration, hypogonadism and growth retardation.[51,52] ATM is held as an inactive dimer in undamaged cells, and even at a very low dose of γ-radiation that causes few DSBs, it undergoes rapid autophosphorylation[53] and is activated in a MRN-dependent manner.[52,54] Intriguingly, ATM is also activated following chromatin alterations or stress response in the absence of DNA DSBs[53] or oxidative response in an MRN-independent manner or by the catalytic inhibition of topoisomerase II.[52,55] Recent studies have uncovered some of the key ATM substrates such as the tumor suppressor protein p53, Nbs1, MDC1, BRCA1, FANCD2 and CHK2 that are essential for the IR-induced cell cycle arrest at G1, S and G2/M stages of the cell cycle.[52]

A newer and not so well-characterized substrate of ATM is the human Pso4.[28] ATM, DNA-PK and ATR are known to phosphorylate their substrates on serine or threonine residues that precede glutamate residues referred to as SQ/TQ motifs.[56] Analysis of the primary amino acid sequence of hPso4 reveal a stretch of amino acids located between positions 144–150 (QAVPSSQPSVVGAG) and 367–373 (TGTMDSQIKIWDLK) that fit the consensus sequence. The serine and glutamine at 149 and 150 are conserved in human, rat and mouse, whereas serine and glutamine at 372 and 373 are conserved in human, rat and *Drosophila* species. A third SQ motif, 270–276 (VVFHPSQDLVFSASP), also appears to be conserved but may not be a good fit for the consensus sequence. Indeed, hPso4 was found to be phosphorylated at S149 in an ATM-dependent manner in response to oxidative stress by hydrogen peroxide and the DNA alkylating agent methyl methane sulfonate.[4] The serine149-phosphorylated hPso4 is predominantly localized in the nucleus, partly protects the cells against apoptosis caused by ICLs or DSB-inducing DNA damage agents and moderately extends the lifespan of human umbilical cord endothelial cells.[4] The equivalent site in yeast Prp19/Pso4, S141, was also uncovered following a quantitative phosphoproteomic approach as a methyl methane sulfonate-induced phosphorylation and a likely target of the ATM/ATR orthologs in yeast, the Mec1/Tel DNA damage checkpoint kinases.[57] As the single-site phosphorylation mutant shows only a partial loss of function, it would be interesting to assess doubly mutated SQ sites (276 and 373) located in the WD40 region of Pso4/SNEV to determine whether these post-translational modifications stabilize the scaffolding or protein–protein interactions of hPso4 with other proteins, especially those of the DNA damage signaling pathways. Moreover, hPso4 protein levels are built up as cells gradually progress during the DSB repair and the kinetics of accumulation varies depending on the nature of the genotoxic insult.[28,45] Increased Prp19/Pso4 expression is able to repress cell proliferation by upregulating p21/CIP1 expression and delay xenograft growth of the lung adenocarcinoma cell line A549 suggestive of its putative role as a tumor suppressor.[58] However, overexpression of BCAS2/SPF27 a member of the Pso4-core complex has been shown to negatively regulate the transcriptional activity of p53.[59] Whether overexpression of BCAS2 is altering the stoichiometry of Pso4-core complex and affecting protein–protein interactions is not known.

These studies suggest that Pso4 is likely to be involved in different stages of repair and may be a recruiting platform for exchange of protein complexes; that is, checkpoint proteins and DNA repair proteins to facilitate repair.[4,28,45–47,60]

hPso4 IN DNA REPLICATION STRESS RESPONSE

Stalling of replication forks activates the ATR/Chk1-dependent replication stress checkpoint pathways.[55] Prolonged stalling of replication forks in cells in the S phase by genotoxic agents such as hydroxy urea, cisplatin or mitomycin C can lead to replication fork collapse, generate single-strand breaks and eventually DSBs to cause genome instability.[61] One of the first proteins that is recruited is the RPA that binds the single-stranded DNA (ssDNA) at the stalled replication forks. This RPA–ssDNA acts as a nucleation point for the recruitment of the DDR checkpoint complex including ATR and its obligate partner, ATRIP. Recently, it has been demonstrated that hPso4/Prp19 colocalizes with proliferating cell nuclear antigen,[49] interacts with the RPA[62,63] and is required for replication fork stability or restart after hydroxyurea-induced DNA damage.[49] It also functions as a component of the damage-sensing complex by interacting with RPA in response to camptothecin (CPT, a topoisomerase I inhibitor), and acts as a sensor of the RPA–ssDNA complex in an ATR-dependent manner.[63] Although phosphorylation of RPA occurs following resection of DSBs induced by CPT, whether ATR stabilizes hPso4–RPA–ssDNA complex by phosphorylation of hPso4 at SQ motifs, especially those in the WD40 domain essential for hPso4–RPA interaction, is not known. Interestingly, a tyrosine residue (Y405) located in the fifth blade of the WD40 domain appears to be essential for interaction with RPA after CPT treatment of cells and for recruitment after DNA damage.[63] hPso4/Prp19 in turn modulates the activity of the DNA replication checkpoint protein ATR in the ATR–ATRIP complex by facilitating autophosphorylation of ATR at Thr1989 and recruitment of ATRIP and thereby phosphorylation of its effectors such as RPA32 and Chk1 but not Chk2 following DNA damage.[62,63] Although the U box of Pso4 with the E3 ubiquitin ligase catalytic activity appeared to be dispensable for interaction with RPA, it was required to promote Chk1 and RPA32 phosphorylations and RPA ubiquitylation. This interaction has been suggested to enhance the recruitment of ATRIP to RPA–ssDNA complex in a DNA damage-dependent manner.[62,63] Indeed, the *pso4-1* mutant initially identified for psoralen sensitivity maps to the U box.[45] Consistent with a role for Pso4/Prp19 in RPA/single-strand break

repair pathway, it was reported to be required for the repair of lesions that arise during replication, replication fork progression and timely S-phase progression in response to replication stalling agents such as CPT and hydroxyurea.[49,62,63]

HUMAN Pso4 INTERACTION WITH TEMPLATE-INDEPENDENT DNA POLYMERASE TdT

Human Pso4 was initially identified as a specific interactor of the BRCT (Breast Cancer Carboxy terminal, BRCA1) domain of the template-independent DNA polymerase, TdT.[28] The BRCT domain is an evolutionarily conserved phosphopeptide-binding motif that recognizes phospho-serine residues,[64] and is found predominantly in proteins participating in DNA metabolic activities, including those functioning in DNA damage response,[65] checkpoint control and cell cycle progression.[66,67] The BRCT domain-containing region of TdT is also essential for interaction with the Ku heterodimer and this interaction with Ku80 is indispensable to add nucleotides in a template-independent manner to increase immunoglobulin and T cell receptor diversity.[68–70] Whether hPso4 has a secondary role in end-joining of DSBs during V(D)J recombination independent of Ku complex, or whether it stimulates the catalytic activity of TdT to promote antigen receptor diversity is not known (Figure 1). In addition, it is unclear whether hPso4 interacts with other BRCT-containing proteins during DNA DSB repair via the ATM-mediated S149 phosphorylation.

Recently, PRP19/PSO4 was also identified in a screen for a role in the alternative NHEJ pathway that is independent of Ku and uses microhomology to repair DSBs.[16,71] Interestingly, this ALT–NHEJ complex appears to promote chromosome translocations in chronic lymphocytic leukemia.[72] The molecular requirement of hPso4/TdT complex in this context, however, remains to be investigated.

HUMAN Pso4 INTERACTION WITH THE NUCLEASE, METNASE

An intriguing aspect of the hPso4 interactome is its requirement as a DNA binding partner for a transposase-like protein called Metnase/SETMAR.[47] This hPso4-interacting transposase-like protein is thought to have originated late in evolution because of the fusion of Hsmar1 transposon downstream of a gene coding for a protein methylase, leading to a functional fusion protein that retained some enzymatic activities, such as a not so robust 5′end processing activity and lack of 3′end processing activity.[73–75] The chimeric protein has the SET domain at its N terminus that harbors the H3K4 and K36 methyl transferase activities, whereas nuclease activity resides in the transposon region. Both these regions are required for its DNA repair activities especially in response to stalled replication forks.[47] Interestingly, the protein *per se* has no transposase activity, although it can bind terminal inverted repeats on double-stranded DNA and displays high specificity for these repeats.[47] This Hsmar1 transposon homology region appears to be required for many of its DNA metabolic activities such as the stable integration of plasmid and viral DNA into the chromosomes, chromosomal decatenation and restart of stalled replication forks in response to CPT and hydroxyurea.[76,77] Initial studies suggested that Metnase–hPso4 interaction facilitates binding of this heteromeric complex to non-terminal inverted repeat DNA sequences. Therefore, it has been suggested that in contrast to the ability of Metnase to bind terminal inverted repeats directly in the absence of its interacting partner, hPso4 may be directing Metnase activity in a sequence-independent manner to facilitate DSB repair.[47,78] Consistent with this notion, hPso4 was found to regulate the recruitment of Metnase to double-stranded DNA ends.[47,78,79]

hPso4 IN DNA ICL REPAIR

Both the yeast Pso4 and its human ortholog appear to be evolutionarily conserved components of pathways required for the repair of DNA ICLs that are profoundly deleterious to cells because of their ability to function as potent blockers of DNA replication fork progression. Conversely, *pso4-1* mutant and PSO4 knockdown cells display exquisite sensitivity to ICLs. In a carefully designed biochemical fractionation experiment using defined plasmid templates containing a single psoralen-induced ICL, Legerski's group[46] has demonstrated a direct role for the hPso4 core complex, including Cdc5L, Spf27 and Plrg1, in the processing of ICLs by reconstituting this reaction *in vitro*. Although hPso4 associates with other members, it displays a very strong affinity for hCdc5, with a dual requirement for both hCdc5 and hPso4 in processing the ICL *in vivo* in plasmid reactivation reporter assays.[46] A clue to how the Pso4 complex may be required in ICL repair comes from the observation that Cdc5L interacted with WRN protein, encoded by a gene defective in Werner Syndrome, that employs a DNA helicase activity for the initial stages of processing of psoralen-induced ICLs.[46] hPso4 has a double-stranded DNA binding activity and is induced in a biphasic manner in response to ICL-induced DNA damage,[28] suggesting the hPso4–Cdc5L complex is perhaps stabilizing the WRN helicase initially at ICLs. Once these ICLs are processed to single-strand gaps or DSBs by endonucleases, hPso4 may act as a docking site for the recruitment of other DDR response pathways that initially do not respond to ICLs. Consistently, Cdc5L has also been shown to interact with ATR and regulate phosphorylation of its effector Chk1 in response to UV-induced DNA damage.[60]

Recently, it has been demonstrated that Pso4/Prp19 associates with a multimeric RNA-binding complex composed of XAB2 and additional proteins to facilitate DNA repair.[80] XAB2 interaction with the xeroderma pigmentosum group A protein, XPA, is enhanced following UV-induced DNA damage. Similarly, XAB2 interaction with RNA Pol II was increased in cells treated with UV and the ICL agents cisplatin and mitomycin C. Consistently, a decrease in XAB2 expression resulted in hypersensitivity to killing by UV light because of an effect on RNA synthesis perhaps caused by a defective RNA splicing.[80]

Whether downregulation of Pso4 in cells affects multiple DNA damage response pathways by affecting mRNA splicing has remained a conundrum. Perturbations in RNA processing, transcription pausing and spliceosome displacement at DNA lesions can lead to genome instability because of the formation of R-loops (RNA/DNA hybrids) that simultaneously expose single-stranded regions that are highly susceptible to breaks.[81] Recently, it has been demonstrated that R loops activate the noncanonical ATM signaling.[82] However, whether loss of Pso4/Prp19 exacerbates R loop formation is not known. Moreover, an RNA splicing-independent role for Prp19/Pso4 as a RPA–ssDNA damage sensor has been reported[63] In Pso4 knockdown cells neither the protein expression of ATM, ATR, ATRIP, DNA-PKcs, BRCA2, MRE11, FANCD2, NBS1, TOPB1,CHK1, CHK2, RPA32 and CDC5L nor the splicing of a panel of pre-mRNAs known to undergo exon skipping when the coupling of splicing and transcription is delinked was affected.[62,63] Consistent with the notion that Pso4 DDR activities are distinct from its role in splicing is the observation that the yeast temperature-sensitive mutant, *pso4-1*, identified for its sensitivity to ICL-induced DNA damage does not display splicing defects in a DNA repair gene containing an intron.[83]

HUMAN Pso4/SNEV IN CELLULAR SENESCENCE

All these above described properties, particularly its role in genome integrity and chromatin homeostasis, may underlie an intriguing aspect of Pso4 functionality—its role in regulating replicative senescence of mammalian cells. It was originally

Table 2. Mutations identified in the *hPSO4* gene in cancers (www.cbioportal.org)

Sample ID	*Cancer study*	*AA change*	*Type*
TCGA-CF-A1HR-01	Bladder (TCGA)	E10K	Missense
TCGA-CU-A3YL-01	Bladder (TCGA)	D40G	Missense
TCGA-BH-A0DP-01	Breast (TCGA)	T287P	Missense
TCGA-BH-A1F5-01	Breast (TCGA)	V182G	Missense
TCGA-BH-A0B6-01	Breast (TCGA)	E196Q	Missense
TCGA-AC-A5EI-01	Breast (TCGA)	S67L	Missense
TCGA-BH-A0DP-01	Breast (TCGA)	T287P	Missense
TCGA-FU-A3TX-01	Cervical (TCGA)	E347D	Missense
TCGA-AA-A01K-01	Colorectal (TCGA)	L29V	Missense
TCGA-AA-3864-01	Colorectal (TCGA)	A219V	Missense
TCGA-AA-3984-01	Colorectal (TCGA)	V481M	Missense
TCGA-76-4931-01	GBM (TCGA)	S392L	Missense
TCGA-HL-7533-01	Head and neck (TCGA)	A491V	Missense
TCGA-AK-3451-01	ccRCC (TCGA)	S492L	Missense
TCGA-B9-5156-01	pRCC (TCGA)	W82L	Missense
H110451	Liver (AMC)	T317A	Missense
LUAD-YINHD	Lung adeno (Broad)	F489L	Missense
LUAD-B02594	Lung adeno (Broad)	Q109[a]	Nonsense
TCGA-73-4662-01	Lung adeno (TCGA)	G476D	Missense
TCGA-64-1678-01	Lung adeno (TCGA)	T287P	Missense
TCGA-56-5898-01	Lung squ (TCGA)	F388L	Missense
TCGA-18-5592-01	Lung squ (TCGA)	E49[a]	Nonsense
TCGA-22-4595-01	Lung squ (TCGA)	E473K	Missense
TCGA-09-2050-01	Ovarian (TCGA)	E197[a]	Nonsense
PR-01-2492	Prostate (Broad/Cornell 2012)	T268S	Missense
TCGA-KC-A4BL-01	Prostate (TCGA)	S220G	Missense
TCGA-G9-6329-01	Prostate (TCGA)	G362[a]	Nonsense
TCGA-EE-A2GM-06	Melanoma (TCGA)	S8F	Missense
TCGA-FS-A1ZK-06	Melanoma (TCGA)	R190K	Missense
YURIF	Melanoma (Yale)	A296V	Missense
YUDEDE	Melanoma (Yale)	E31K	Missense
pfg043T	Stomach (Pfizer UHK)	L363H	Missense
pfg062T	Stomach (Pfizer UHK)	F427C	Missense
TCGA-BR-8363-01	Stomach (TCGA)	R100H	Missense
TCGA-BR-8591-01	Stomach (TCGA)	S8fs	FS del
TCGA-BR-4184-01	Stomach (TCGA)	T354A	Missense
TCGA-BR-8680-01	Stomach (TCGA)	R422H	Missense
TCGA-BR-4184-01	Stomach (TCGA)	R128Q	Missense
TCGA-B5-A11Q-01	Uterine (TCGA)	A242V	Missense
TCGA-AP-A059-01	Uterine (TCGA)	Q94H	Missense
TCGA-AP-A051-01	Uterine (TCGA)	I290V	Missense
TCGA-D1-A103-01	Uterine (TCGA)	R382H	Missense
TCGA-BS-A0UA-01	Uterine (TCGA)	V247I	Missense
TCGA-B5-A11E-01	Uterine (TCGA)	G368[a]	Nonsense
TCGA-D1-A15X-01	Uterine (TCGA)	H263N	Missense

Abbreviations: AA, amino acid; AMC, Asan Medical Center; ccRCC, clear cell renal cell carcinoma; GBM, glioblastoma multiforme; Lung adeno, lung adenocarcinoma; Lung squ, lung squamous cell carcinoma; pRCC, papillary renal cell carcinoma; TCGA, The Cancer Genome Atlas. [a]Nonsense mutation.

identified as SNEV that was downregulated in aged human umbilical cord endothelial cells.[29] Conversely, the replicative potential was restored by overexpression of Pso4. Consistently, genetic deletion of SNEV/PSO4 in mice is embryonic lethal.[84] Cells derived from heterozygous mice display properties of reduced population doublings and premature senescence. Its role in promoting replicative capacity appears to be reflected more profoundly in the self-renewal potential of hematopoietic stem cells.[85] Although SNEV mRNA expression was high in lineage-depleted Lin(−) precursor cells of the bone marrow and in the progenitor cell subset with highest colony-forming ability and self-renewal capacity (Lin(−)/Sca-1(+), the differences were not as pronounced after cells had progressed further in differentiation.[85] Conversely, Lin(−) cells derived from heterozygous SNEV knockout mice formed fewer primary and secondary colonies following stimulation with granulocyte-macrophage colony-stimulating factor, a defect that was rescued by ectopic SNEV expression.[85] These phenotypic outcomes could be perhaps attributed to cells accumulating DNA damage in the form of unrepaired breaks and broken chromosomes, eventually leading to genome instability. The contribution of DDR to genome instability and aging has been observed for other DNA repair factors.[86] Consistently, a recent study demonstrates that knockdown of BCAS2 destabilizes Pso4/Prp19-BCAS2/Spf27 core complex in human cells.[87] Mechanistically, this complex appears to regulate spindle assembly by association with the spindle assembly factor in Xenopus egg extracts to facilitate proper formation of mitotic spindles and ensure proper metaphase alignment of the chromosomes.[87]

PSO4 IN CANCER

Human Pso4 mRNA expression is seen across several tissue types.[28] The gene is located on chromosome 11q12.2 in humans. Rare instances of mutations—truncating, missense and in-frame mutations—have been identified to be dispersed throughout the *PSO4* gene in several primary cancers and cancer-derived cell lines (Table 2). Striking among these are the truncating mutations leading to complete loss of function in lung squamous cell carcinoma at the E49 residue in the U box, Q109 in the Prp19 homology region and two adjacent truncations, G362 in prostate adenocarcinoma and G368 in endometrial carcinoma in the third WD40 repeat.[88,89] In addition, cancer-specific mutations have been observed within ~70 amino acid U box of PSO4, such as the S8F, E10K and a D40G located next to a highly conserved proline residue (P41) and R422H, an evolutionarily conserved residue in the fifth blade of the WD40 repeat.[32,89] Deletions or mutations in the U box have been found to affect the ubiquitination activity of E3 ligases.[90] The impact of cancer-specific mutations on the function of PSO4 will be critical to understand its pleiotropic roles in maintaining genome stability and tumor suppressive activity.[58]

Mixed lineage leukemia displays the MLL gene fused with an eclectic mix of other genes.[91] Interestingly, chromosome 11 harbors both *PSO4/PRP19* and the *MLL* in humans. DSB-induced recombination events appear to be frequent within chromosome 11, leading to inversions or deletions on 11p or 11q (Inv, Del).[91] One such event that has been recently identified in a leukemia patient is the fusion of *MLL* with *PRP19*. Whether this is a recurrent pathological gene fusion event in multiple MLL patient samples that generates a fusion protein that harbors the sequence-independent DNA binding property of PSO4 and activating methyl transferase activity of MLL remains to be seen. Interestingly, this MLL–PRP19 fusion translocation appears to be reminiscent of the recently described Pso4/Metnase complex that couples the DNA binding and methyl transferase activities in DSB repair.[47]

CONCLUSIONS

Genetic, biochemical and structural studies on Pso4/Prp19 have yielded rich clues into how this ubiquitously expressed and essential protein employs its enzymatic and oligomerization capacity to participate as an integral component of complex molecular machineries and simultaneously maintain a certain degree of fluidity to fulfill its various roles. However, compared with many other proteins implicated in the DDR pathways, PSO4/PRP19 has been fairly underexplored. It is still unclear how the Pso4/Prp19 core complex is essential for maintaining levels of the tumor suppressor BRCA1.[49] Moreover, its contribution to antigen receptor diversity during V(D)J recombination, as a TdT interacting partner, has remained elusive. Conditional knockout mouse models of Pso4 or inducible CRISPR/Cas9-based gene knockout studies in lymphocytes should allow us to address the

requirement of PSO4 as an essential gene in this important area. Whether targeting this protein in cancer cells, particularly cancer stem cells, can lead to an earlier onset of replicative senescence by triggering genome instability remains to be seen. Thus, the current knowledge of Pso4 provides a solid fundamental basis for future studies on its role in chemoresistance, radioresistance and genome instability of cancer stem cells.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ACKNOWLEDGEMENTS

I thank Dr Nupam Mahajan and Professor William Kaufmann (UNC Chapel Hill) for critical reading of the manuscript and suggestions. I appreciate Urvashi Mahajan for help with the illustrations. This work is supported in part by Department of Defense Awards W81XWH-12-1-0248, W81XWH-14-1-0251 and W81XWH-15-1-0059 to KM.

REFERENCES

1 d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T *et al.* A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 2003; **426**: 194–198.
2 von Zglinicki T, Saretzki G, Ladhoff J, d'Adda di Fagagna F, Jackson SP. Human cell senescence as a DNA damage response. *Mech Ageing Dev* 2005; **126**: 111–117.
3 Garinis GA, van der Horst GT, Vijg J, Hoeijmakers JH. DNA damage and ageing: new-age ideas for an age-old problem. *Nat Cell Biol* 2008; **10**: 1241–1247.
4 Dellago H, Khan A, Nussbacher M, Gstraunthaler A, Lammermann I, Schosserer M *et al.* ATM-dependent phosphorylation of SNEVhPrp19/hPso4 is involved in extending cellular life span and suppression of apoptosis. *Aging* 2012; **4**: 290–304.
5 Reinhardt HC, Schumacher B. The p53 network: cellular and systemic DNA damage responses in aging and cancer. *Trends Genet* 2012; **28**: 128–136.
6 Jiang G, Plo I, Wang T, Rahman M, Cho JH, Yang E *et al.* BRCA1-Ku80 protein interaction enhances end-joining fidelity of chromosomal double-strand breaks in the G1 phase of the cell cycle. *J Biol Chem* 2013; **288**: 8966–8976.
7 Gupta GP, Vanness K, Barlas A, Manova-Todorova KO, Wen YH, Petrini JH. The Mre11 complex suppresses oncogene-driven breast tumorigenesis and metastasis. *Mol Cell* 2013; **52**: 353–365.
8 Lee JH, Paull TT. Activation and regulation of ATM kinase activity in response to DNA double-strand breaks. *Oncogene* 2007; **26**: 7741–7748.
9 Lammens K, Bemeleit DJ, Mockel C, Clausing E, Schele A, Hartung S *et al.* The Mre11:Rad50 structure shows an ATP-dependent molecular clamp in DNA double-strand break repair. *Cell* 2011; **145**: 54–66.
10 Lee BS, Gapud EJ, Zhang S, Dorsett Y, Bredemeyer A, George R *et al.* Functional intersection of ATM and DNA-dependent protein kinase catalytic subunit in coding end joining during V(D)J recombination. *Mol Cell Biol* 2013; **33**: 3568–3579.
11 Zha S, Jiang W, Fujiwara Y, Patel H, Goff PH, Brush JW *et al.* Ataxia telangiectasia-mutated protein and DNA-dependent protein kinase have complementary V(D)J recombination functions. *Proc Natl Acad Sci USA* 2011; **108**: 2028–2033.
12 Perkins EJ, Nair A, Cowley DO, Van Dyke T, Chang Y, Ramsden DA. Sensing of intermediates in V(D)J recombination by ATM. *Genes Dev* 2002; **16**: 159–164.
13 Zha S, Guo C, Boboila C, Oksenych V, Cheng HL, Zhang Y *et al.* ATM damage response and XLF repair factor are functionally redundant in joining DNA breaks. *Nature* 2011; **469**: 250–254.
14 Kakarougkas A, Jeggo PA. DNA DSB repair pathway choice: an orchestrated handover mechanism. *Br J Radiol* 2014; **87**: 20130685.
15 Motoyama N, Naka K. DNA damage tumor suppressor genes and genomic instability. *Curr Opin Genet Dev* 2004; **14**: 11–16.
16 Howard SM, Yanez DA, Stark JM. DNA damage response factors from diverse pathways, including DNA crosslink repair, mediate alternative end joining. *PLoS Genet* 2015; **11**: e1004943.
17 Mateos-Gomez PA, Gong F, Nair N, Miller KM, Lazzerini-Denchi E, Sfeir A. Mammalian polymerase theta promotes alternative NHEJ and suppresses recombination. *Nature* 2015; **518**: 254–257.
18 Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, Petalcorin MI *et al.* Homologous-recombination-deficient tumours are dependent on Poltheta-mediated repair. *Nature* 2015; **518**: 258–262.
19 Vermeij WP, Hoeijmakers JH, Pothof J. Aging: not all DNA damage is equal. *Curr Opin Genet Dev* 2014; **26**: 124–130.
20 Sykora P, Misiak M, Wang Y, Ghosh S, Leandro GS, Liu D *et al.* DNA polymerase beta deficiency leads to neurodegeneration and exacerbates Alzheimer disease phenotypes. *Nucleic Acids Res* 2015; **43**: 943–959.
21 Iyama T, Lee SY, Berquist BR, Gileadi O, Bohr VA, Seidman MM *et al.* CSB interacts with SNM1A and promotes DNA interstrand crosslink processing. *Nucleic Acids Res* 2015; **43**: 247–258.
22 Kolodner RD, Putnam CD, Myung K. Maintenance of genome stability in Saccharomyces cerevisiae. *Science* 2002; **297**: 552–557.
23 Myung K, Kolodner RD. Suppression of genome instability by redundant S-phase checkpoint pathways in Saccharomyces cerevisiae. *Proc Natl Acad Sci USA* 2002; **99**: 4500–4507.
24 Jaehnig EJ, Kuo D, Hombauer H, Ideker TG, Kolodner RD. Checkpoint kinases regulate a global network of transcription factors in response to DNA damage. *Cell Rep* 2013; **4**: 174–188.
25 da Silva KV, de Morais Junior MA, Henriques JA. The PSO4 gene of S. cerevisiae is important for sporulation and the meiotic DNA repair of photoactivated psoralen lesions. *Curr Genet* 1995; **27**: 207–212.
26 Grey M, Dusterhoft A, Henriques JA, Brendel M. Allelism of PSO4 and PRP19 links pre-mRNA processing with recombination and error-prone DNA repair in Saccharomyces cerevisiae. *Nucleic Acids Res* 1996; **24**: 4009–4014.
27 Gotzmann J, Gerner C, Meissner M, Holzmann K, Grimm R, Mikulits W *et al.* hNMP 200: a novel human common nuclear matrix protein combining structural and regulatory functions. *Exp Cell Res* 2000; **261**: 166–179.
28 Mahajan KN, Mitchell BS. Role of human Pso4 in mammalian DNA repair and association with terminal deoxynucleotidyl transferase. *Proc Natl Acad Sci USA* 2003; **100**: 10746–10751.
29 Grillari J, Ajuh P, Stadler G, Loscher M, Voglauer R, Ernst W *et al.* SNEV is an evolutionarily conserved splicing factor whose oligomerization is necessary for spliceosome assembly. *Nucleic Acids Res* 2005; **33**: 6868–6883.
30 Chan SP, Kao DI, Tsai WY, Cheng SC. The Prp19p-associated complex in spliceosome activation. *Science* 2003; **302**: 279–282.
31 Vander Kooi CW, Ohi MD, Rosenberg JA, Oldham ML, Newcomer ME, Gould KL *et al.* The Prp19 U-box crystal structure suggests a common dimeric architecture for a class of oligomeric E3 ubiquitin ligases. *Biochemistry* 2006; **45**: 121–130.
32 Vander Kooi CW, Ren L, Xu P, Ohi MD, Gould KL, Chazin WJ. The Prp19 WD40 domain contains a conserved protein interaction region essential for its function. *Structure* 2010; **18**: 584–593.
33 Ohi MD, Vander Kooi CW, Rosenberg JA, Ren L, Hirsch JP, Chazin WJ *et al.* Structural and functional analysis of essential pre-mRNA splicing factor Prp19p. *Mol Cell Biol* 2005; **25**: 451–460.
34 Aravind L, Iyer LM, Koonin EV. Scores of RINGS but no PHDs in ubiquitin signaling. *Cell Cycle* 2003; **2**: 123–126.
35 Ohi MD, Vander Kooi CW, Rosenberg JA, Chazin WJ, Gould KL. Structural insights into the U-box, a domain associated with multi-ubiquitination. *Nat Struct Biol* 2003; **10**: 250–255.
36 Grote M, Wolf E, Will CL, Lemm I, Agafonov DE, Schomburg A *et al.* Molecular architecture of the human Prp19/CDC5L complex. *Mol Cell Biol* 2010; **30**: 2105–2119.
37 Tarn WY, Hsu CH, Huang KT, Chen HR, Kao HY, Lee KR *et al.* Functional association of essential splicing factor(s) with PRP19 in a protein complex. *EMBO J* 1994; **13**: 2421–2431.
38 Song EJ, Werner SL, Neubauer J, Stegmeier F, Aspden J, Rio D *et al.* The Prp19 complex and the Usp4Sart3 deubiquitinating enzyme control reversible ubiquitination at the spliceosome. *Genes Dev* 2010; **24**: 1434–1447.
39 Makarova OV, Makarov EM, Urlaub H, Will CL, Gentzel M, Wilm M *et al.* A subset of human 35S U5 proteins, including Prp19, function prior to catalytic step 1 of splicing. *EMBO J* 2004; **23**: 2381–2391.
40 Tarn WY, Lee KR, Cheng SC. Yeast precursor mRNA processing protein PRP19 associates with the spliceosome concomitant with or just after dissociation of U4 small nuclear RNA. *Proc Natl Acad Sci USA* 1993; **90**: 10821–10825.
41 Chan SP, Cheng SC. The Prp19-associated complex is required for specifying interactions of U5 and U6 with pre-mRNA during spliceosome activation. *J Biol Chem* 2005; **280**: 31190–31199.
42 David CJ, Boyne AR, Millhouse SR, Manley JL. The RNA polymerase II C-terminal domain promotes splicing activation through recruitment of a U2AF65-Prp19 complex. *Genes Dev* 2011; **25**: 972–983.
43 Lu X, Legerski RJ. The Prp19/Pso4 core complex undergoes ubiquitylation and structural alterations in response to DNA damage. *Biochem Biophys Res Commun* 2007; **354**: 968–974.
44 Marin I. Ancient origin of animal U-box ubiquitin ligases. *BMC Evol Biol* 2010; **10**: 331.
45 Legerski RJ. The Pso4 complex splices into the DNA damage response. *Cell Cycle* 2009; **8**: 3448–3449.
46 Zhang N, Kaur R, Lu X, Shen X, Li L, Legerski RJ. The Pso4 mRNA splicing and DNA repair complex interacts with WRN for processing of DNA interstrand cross-links. *J Biol Chem* 2005; **280**: 40559–40567.
47 Beck BD, Park SJ, Lee YJ, Roman Y, Hromas RA, Lee SH. Human Pso4 is a metnase (SETMAR)-binding partner that regulates metnase function in DNA repair. *J Biol Chem* 2008; **283**: 9023–9030.

48 de Andrade HH, Marques EK, Schenberg AC, Henriques JA. The PSO4 gene is responsible for an error-prone recombinational DNA repair pathway in Saccharomyces cerevisiae. *Mol Gen Genet* 1989; **217**: 419–426.
49 Abbas M, Shanmugam I, Bsaili M, Hromas R, Shaheen M. The role of the human psoralen 4 (hPso4) protein complex in replication stress and homologous recombination. *J Biol Chem* 2014; **289**: 14009–14019.
50 Alvarez-Quilon A, Serrano-Benitez A, Lieberman JA, Quintero C, Sanchez-Gutierrez D, Escudero LM *et al.* ATM specifically mediates repair of double-strand breaks with blocked DNA ends. *Nat Commun* 2014; **5**: 3347.
51 Lim DS, Kim ST, Xu B, Maser RS, Lin J, Petrini JH *et al.* ATM phosphorylates p95/nbs1 in an S-phase checkpoint pathway. *Nature* 2000; **404**: 613–617.
52 Paull TT. Mechanisms of ATM Activation. *Annu Rev Biochem* 2015; **84**: 711–738.
53 Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003; **421**: 499–506.
54 Berkovich E, Monnat Jr RJ, Kastan MB. Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. *Nat Cell Biol* 2007; **9**: 683–690.
55 Kaufmann WK. The human intra-S checkpoint response to UVC-induced DNA damage. *Carcinogenesis* 2010; **31**: 751–765.
56 Kim ST, Lim DS, Canman CE, Kastan MB. Substrate specificities and identification of putative substrates of ATM kinase family members. *J Biol Chem* 1999; **274**: 37538–37543.
57 Smolka MB, Albuquerque CP, Chen SH, Zhou H. Proteome-wide identification of in vivo targets of DNA damage checkpoint kinases. *Proc Natl Acad Sci USA* 2007; **104**: 10364–10369.
58 Benjamin AB, Zhou X, Isaac O, Zhao H, Song Y, Chi X *et al.* PRP19 upregulation inhibits cell proliferation in lung adenocarcinomas by p21-mediated induction of cell cycle arrest. *Biomed Pharmacother* 2014; **68**: 463–470.
59 Kuo PC, Tsao YP, Chang HW, Chen PH, Huang CW, Lin ST *et al.* Breast cancer amplified sequence 2, a novel negative regulator of the p53 tumor suppressor. *Cancer Res* 2009; **69**: 8877–8885.
60 Zhang N, Kaur R, Akhter S, Legerski RJ. Cdc5L interacts with ATR and is required for the S-phase cell-cycle checkpoint. *EMBO Rep* 2009; **10**: 1029–1035.
61 Zhang J, Dewar JM, Budzowska M, Motnenko A, Cohn MA, Walter JC. DNA interstrand cross-link repair requires replication-fork convergence. *Nat Struct Mol Biol* 2015; **22**: 242–247.
62 Wan L, Huang J. The PSO4 protein complex associates with replication protein A (RPA) and modulates the activation of ataxia telangiectasia-mutated and Rad3-related (ATR). *J Biol Chem* 2014; **289**: 6619–6626.
63 Marechal A, Li JM, Ji XY, Wu CS, Yazinski SA, Nguyen HD *et al.* PRP19 transforms into a sensor of RPA-ssDNA after DNA damage and drives ATR activation via a ubiquitin-mediated circuitry. *Mol Cell* 2014; **53**: 235–246.
64 Rodriguez M, Yu X, Chen J, Songyang Z. Phosphopeptide binding specificities of BRCA1 COOH-terminal (BRCT) domains. *J Biol Chem* 2003; **278**: 52914–52918.
65 Koonin EV, Altschul SF, Bork P. BRCA1 protein productsy Functional motifs. *Nat Genet* 1996; **13**: 266–268.
66 Yu X, Chini CC, He M, Mer G, Chen J. The BRCT domain is a phospho-protein binding domain. *Science* 2003; **302**: 639–642.
67 Woods NT, Mesquita RD, Sweet M, Carvalho MA, Li X, Liu Y *et al.* Charting the landscape of tandem BRCT domain-mediated protein interactions. *Sci Signal* 2012; **5**: rs6.
68 Mahajan KN, Gangi-Peterson L, Sorscher DH, Wang J, Gathy KN, Mahajan NP *et al.* Association of terminal deoxynucleotidyl transferase with Ku. *Proc Natl Acad Sci USA* 1999; **96**: 13926–13931.
69 Gangi-Peterson L, Sorscher DH, Reynolds JW, Kepler TB, Mitchell BS. Nucleotide pool imbalance and adenosine deaminase deficiency induce alterations of N-region insertions during V(D)J recombination. *J Clin Invest* 1999; **103**: 833–841.
70 Purugganan MM, Shah S, Kearney JF, Roth DB. Ku80 is required for addition of N nucleotides to V(D)J recombination junctions by terminal deoxynucleotidyl transferase. *Nucleic Acids Res* 2001; **29**: 1638–1646.
71 Bennardo N, Cheng A, Huang N, Stark JM. Alternative-NHEJ is a mechanistically distinct pathway of mammalian chromosome break repair. *PLoS Genet* 2008; **4**: e1000110.
72 Tobin LA, Robert C, Rapoport AP, Gojo I, Baer MR, Tomkinson AE *et al.* Targeting abnormal DNA double-strand break repair in tyrosine kinase inhibitor-resistant chronic myeloid leukemias. *Oncogene* 2013; **32**: 1784–1793.
73 Cordaux R, Udit S, Batzer MA, Feschotte C. Birth of a chimeric primate gene by capture of the transposase gene from a mobile element. *Proc Natl Acad Sci USA* 2006; **103**: 8101–8106.
74 Liu D, Bischerour J, Siddique A, Buisine N, Bigot Y, Chalmers R. The human SETMAR protein preserves most of the activities of the ancestral Hsmar1 transposase. *Mol Cell Biol* 2007; **27**: 1125–1132.
75 Roman Y, Oshige M, Lee YJ, Goodwin K, Georgiadis MM, Hromas RA *et al.* Biochemical characterization of a SET and transposase fusion protein, Metnase: its DNA binding and DNA cleavage activity. *Biochemistry* 2007; **46**: 11369–11376.
76 Wray J, Williamson EA, Royce M, Shaheen M, Beck BD, Lee SH *et al.* Metnase mediates resistance to topoisomerase II inhibitors in breast cancer cells. *PLoS One* 2009; **4**: e5323.
77 Williamson EA, Rasila KK, Corwin LK, Wray J, Beck BD, Severns V *et al.* The SET and transposase domain protein Metnase enhances chromosome decatenation: regulation by automethylation. *Nucleic Acids Res* 2008; **36**: 5822–5831.
78 Beck BD, Lee SS, Williamson E, Hromas RA, Lee SH. Biochemical characterization of metnase's endonuclease activity and its role in NHEJ repair. *Biochemistry* 2011; **50**: 4360–4370.
79 Beck BD, Lee SS, Hromas R, Lee SH. Regulation of Metnase's TIR binding activity by its binding partner, Pso4. *Arch Biochem Biophys* 2010; **498**: 89–94.
80 Kuraoka I, Ito S, Wada T, Hayashida M, Lee L, Saijo M *et al.* Isolation of XAB2 complex involved in pre-mRNA splicing, transcription, and transcription-coupled repair. *J Biol Chem* 2008; **283**: 940–950.
81 Chan YA, Hieter P, Stirling PC. Mechanisms of genome instability induced by RNA-processing defects. *Trends Genet* 2014; **30**: 245–253.
82 Tresini M, Warmerdam DO, Kolovos P, Snijder L, Vrouwe MG, Demmers JA *et al.* The core spliceosome as target and effector of non-canonical ATM signalling. *Nature* 2015; **523**: 53–58.
83 Henriques JA, Vicente EJ, Leandro da Silva KV, Schenberg AC. PSO4: a novel gene involved in error-prone repair in Saccharomyces cerevisiae. *Mut Res* 1989; **218**: 111–124.
84 Fortschegger K, Wagner B, Voglauer R, Katinger H, Sibilia M, Grillari J. Early embryonic lethality of mice lacking the essential protein SNEV. *Mol Cell Biol* 2007; **27**: 3123–3130.
85 Schraml E, Voglauer R, Fortschegger K, Sibilia M, Stelzer I, Grillari J *et al.* Haploinsufficiency of senescence evasion factor causes defects of hematopoietic stem cells functions. *Stem Cells Dev* 2008; **17**: 355–366.
86 Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 2007; **447**: 725–729.
87 Hofmann JC, Tegha-Dunghu J, Drager S, Will CL, Luhrmann R, Gruss OJ. The Prp19 complex directly functions in mitotic spindle assembly. *PLoS One* 2013; **8**: e74851.
88 Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal* 2013; **6**: pl1.
89 Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA *et al.* The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov* 2012; **2**: 401–404.
90 Hatakeyama S, Yada M, Matsumoto M, Ishida N, Nakayama KI. U box proteins as a new family of ubiquitin-protein ligases. *J Biol Chem* 2001; **276**: 33111–33120.
91 Meyer C, Hofmann J, Burmeister T, Groger D, Park TS, Emerenciano M *et al.* The MLL recombinome of acute leukemias in 2013. *Leukemia* 2013; **27**: 2165–2176.
92 Kleinridders A, Pogoda HM, Irlenbusch S, Smyth N, Koncz C, Hammerschmidt M *et al.* PLRG1 is an essential regulator of cell proliferation and apoptosis during vertebrate development and tissue homeostasis. *Mol Cell Biol* 2009; **29**: 3173–3185.
93 Grillari J, Loscher M, Denegri M, Lee K, Fortschegger K, Eisenhaber F *et al.* Blom7alpha is a novel heterogeneous nuclear ribonucleoprotein K homology domain protein involved in pre-mRNA splicing that interacts with SNEVPrp19-Pso4. *J Biol Chem* 2009; **284**: 29193–29204.
94 Loscher M, Fortschegger K, Ritter G, Wostry M, Voglauer R, Schmid JA *et al.* Interaction of U-box E3 ligase SNEV with PSMB4, the beta7 subunit of the 20S proteasome. *Biochem J* 2005; **388**: 593–603.
95 Dellago H, Loscher M, Ajuh P, Ryder U, Kaisermayer C, Grillari-Voglauer R *et al.* Exo70, a subunit of the exocyst complex, interacts with SNEV(hPrp19/hPso4) and is involved in pre-mRNA splicing. *Biochem J* 2011; **438**: 81–91.