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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
The genetic evolution of normal breast epithelial cells into cancer cells is largely determined by the fidelity of DNA replication, repair, and cell cycle division. The control mechanisms that restrain cell cycle transition after DNA damage are known as cell cycle checkpoints. Loss or attenuation of checkpoint function can result in gene mutations, chromosome damage, and aneuploidy, all of which can contribute to breast tumorigeneiss. This proposal focuses on the G2 cell cycle checkpoint signaling pathway in breast epithelial cells and how one component of this checkpoint pathway, the Chk1 kinase, may offer the potential for therapeutic intervention. The objective of the proposal is to test the following two-part hypothesis. First, Chk1 kinase is activated by genotoxic stress and its activity is required for G2 checkpoint function in breast epithelial cells. Second, ablation of Chk1 function in mammary epithelial cells will ablate G2 checkpoint function and sensitize cells to anticancer agents that induce DNA damage. The following specific aims are proposed to test this hypothesis: (1) To determine how Chk1 is regulated after exposure of human breast epithelial cells to anticancer agents. (2) To determine if conditional knock-out of Chk1 in mouse mammary epithelial cells abrogates G2 checkpoint function and sensitizes mammary epithelial cells to currently used anticancer agents.

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INTRODUCTION

Breast cancer continues to be the leading cause of death in American women between 30 and 70 years of age and approximately 1 in 10 women will develop breast cancer during their lifetime. Thus, the following must continue to be a high priority: basic research focused on determining the molecular basis of breast cancer and translation of this information to new treatments that will exploit the molecular defects in breast cancer cells. Most human breast tumors arise from multiple genetic changes which gradually transform growth-limited cells into highly invasive cells that are unresponsive to growth controls. The genetic evolution of normal breast epithelial cells into cancer cells is largely determined by the fidelity of DNA replication, repair, and division. Cell cycle arrest in response to DNA damage is integral to the maintenance of genomic integrity. The control mechanisms that restrain cell cycle transition after DNA damage are known as cell cycle checkpoints. Loss or attenuation of checkpoint function can result in gene mutations, chromosome damage, and aneuploidy, all of which can contribute to breast tumorigenesis. Recent evidence from our laboratory and others suggest that tumor cells with defective DNA damage cell cycle checkpoint function have increased sensitivity to anticancer agents. Therefore, a further understanding of the biochemical pathways that mediate checkpoint function may lead to the identification of more effective breast cancer treatments.

This proposal focuses on the G2 cell cycle checkpoint signaling pathway in breast epithelial cells and how one component of this checkpoint pathway, the Chk1 kinase, may offer the potential for therapeutic intervention. The following two-part hypothesis is put forth. First, Chk1 kinase is activated by genotoxic stress and its activity is required for G2 checkpoint function in breast epithelial cells. Second, ablation of Chk1 function in mammary epithelial cells will ablate G2 checkpoint function and sensitize cells to anticancer agents that induce DNA damage. The following specific aims are proposed to test this hypothesis: (1) To determine how Chk1 is regulated after exposure of human breast epithelial cells to anticancer agents. (2) To determine if conditional knock-out of Chk1 in mouse mammary epithelial cells abrogates G2 checkpoint function and sensitizes mammary epithelial cells to currently used anticancer agents.

BODY

A description of the research accomplishments associated with each Task, outlined in the approved Statement of Work, is provided below. Please note, the postdoctoral fellow that was hired for the position had a change in relocation plans, due to family reasons, and did not take the position offered in Fall of 2001. Thus, the PI initiated a new search and hired a senior postdoctoral fellow, who brings a significant level of experience in the area of cellular kinase biochemistry to the project. The biosketch of this individual, Dr. Eugenia Yazlovitskaya, is included in the Appendix. She started her position at Vanderbilt, July 1, 2002. As a result of this recruitment issue, there has been a slight delay in acquisition of data from the Tasks, although we have already made progress in several assays and reagent development as outlined below.

STATEMENT OF WORK

Task 1. To determine how Chk1 is regulated after exposure of human breast epithelial cells to anticancer agents.

a. Analyze Chk1 phosphorylation status after treatment of cells with anticancer agent (Months 1-12)

Using protein biochemistry techniques, in particular, 1-dimensional and 2-dimensional gel electrophoresis, we are determining how many phospho-forms of Chk1 exist in both breast tumor-derived cell lines (MCF-7, MDA-MB-468), as well as a non-transformed cell line (MCF-10A) and primary cultures of normal human mammary epithelial cells. We are examining Chk1 protein from cells grown under control or anticancer agent-treated conditions. We believe the use of normal breast epithelial cells is of great importance since it is necessary to dissect the normal pathways to know what is altered and what can be manipulated in cancer cells for therapeutic intervention. We are considering mapping the phosphorylation sites on Chk1 using mass spectrometric (MS) techniques and will explore this further in the coming year. MS would be the most straightforward approach for determining residues that are phosphorylated; however, the most technically challenging.

b. Determine Chk1 subcellular localization after treatment of cells with anticancer agents (Months 1-12)

After determining which cells discussed above have the most robust phosphorylation, we will proceed with subcellular localization of various phospho-forms of the protein. We have already worked out conditions for separating nuclear, cytoplasmic fractions, and membrane fractions from various epithelial cells. Further, we have good markers to verify these fractions, including myc, jun, actin, PIG3, ras, and EGF receptor.

c. Analyze Chk1 kinase activity after treatment of cells with anticancer agents (Months 1-12)

We have made significant progress in this area already with development of an *in vitro* Chk1 kinase assay using a Cdc25C peptide that contains a serine at 216 that can be phosphorylated by Chk1. We generated this peptide as a fusion protein in *E. Coli.* Further, for a control, we

generated a recombinant peptide that contains an alanine at amino acid 216, resulting in a peptide that is not phosphorylated. We have this assay up and running and have already analyzed the activity of immunoprecipitated Chk1 from non-transformed and tumor-derived breast epithelial cells under control conditions and after treatment with cisplatin, adriamycin, and 5-FU. Further, we are extending our studies to Taxol as well since there have been reports indicating that Taxol can induce DNA damage secondarily through spindle alteration and this agent is a leading anticancer treatment for breast cancer.

Task 2. To determine if conditional knock-out of Chk1 in mouse mammary epithelial cells abrogates G2 checkpoint function and sensitizes mammary epithelial cells to currently used anticancer agents.

a. Generation and analysis of mice in which Chk1 kinase is conditionally expressed in the mammary gland (Months 1-36)

We have the mouse Chk1 gene and a good plan for use of the latest Cre-lox targeting vectors to allow spatial and temporal knock-out of Chk1 in the mammary gland. We have also begun using a vector called pSUPER that will express Chk1 RNAi (inhibitory RNA molecules that will target endogenous Chk1 transcript for degradation). Use of pSUPER-Chk1 will allow us to perform somatic cell knock-outs in both non-transformed and tumor-derived breast cancer cells and analyze the result of ablation of Chk1 activity in cellular response to anticancer treatments. We can directly test the second part of our hypothesis, albeit at the single cell level, if we have success with this latter approach. However, the generation of the mice will confirm our *in vitro* observations and allow us to test our hypothesis in a three-dimensional model of the mammary gland which most closely recapitulates the human situation.

KEY RESEARCH ACCOMPLISHMENTS

•Development of a Chk1 kinase assay to analyze the effect of various anticancer agents on Chk1 activity.

•Initial development of a 2D isoelectric focusing assay to analyze Chk1 phosphorylation status in protein lysates prepared from control and anticancer agent treated non-transformed and transformed breast epithelial cells.

REPORTABLE OUTCOMES

An abstract for the 2002 Era of Hope Meeting in Orlando, FL.

Stewart, Z.A. and Pietenpol, J.A. (2002) Cell Cycle Dysregulation and Anticancer Therapy. *Trends in Pharmacologic Sciences*. In Press.

CONCLUSIONS

The ultimate goal of this study is to determine the role Chk1 kinase plays in (i) cell cycle checkpoint response and (ii) if the kinase represents a valid target for rational drug and therapy design. Understanding the function of Chk1 will enhance our knowledge of the checkpoint controls involved in preventing transformation of normal breast epithelial cells, and may eventually lead to development of novel therapeutic treatments for breast cancer patients.

APPENDIX

Contents:

One publication:
Stewart, Z.A. and Pietenpol, J.A. (2002) Cell Cycle Dysregulation and Anticancer Therapy. *Trends in Pharmacologic Sciences*. In Press.

One Biographical Sketch:
Eugenia Yazlovitskaya, Ph.D.

Cell Cycle Dysregulation and Anticancer Therapy

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SUMMARY

Cell cycle dysregulation is a hallmark of tumor cells. The ability of normal cells to undergo cell cycle arrest after DNA damage is critical for maintenance of genomic integrity. The biochemical pathways that mediate cell cycle transitions in response to cellular stressors are called checkpoints. Defective checkpoint function results in genetic modifications that contribute to tumorigenesis. Regulation of checkpoint signaling also has important clinical implications, as abrogation of checkpoint function can enhance tumor cell sensitivity to chemotherapeutics. This review will focus on current anticancer therapies that target checkpoint signaling pathways, as well as strategies for the development of novel chemotherapeutic agents.

Keywords: cell cycle, checkpoint, G1/S, G2/M, anticancer

Abbreviations: APC, anaphase-promoting complex; CDK, cyclin-dependent kinase; CDKI, cyclin-dependent kinase inhibitor; NCI, National Cancer Institute; p16, p16INK4A; p21, p21Waf1/Cip1; pRB, retinoblastoma protein

THE CELL CYCLE MACHINERY

Eukaryotic cells have evolved signaling pathways to coordinate cell cycle transitions and ensure faithful replication of the genome prior to cell division. Cell cycle progression is stimulated by protein kinase complexes, each consisting of a cyclin and a cyclin-dependent kinase (CDK) [1]. CDK expression is constitutive throughout the cell cycle, while cyclin protein expression is restricted by transcriptional regulation of cyclin genes and ubiquitin-mediated degradation of cyclin proteins [2]. CDK activation requires binding a cyclin partner, as well as site-specific phosphorylation [1].

The cyclin D1/CDK4,6, cyclin E/CDK2, and cyclin A/CDK2 complexes regulate G1- and S-phase progression [1]. The retinoblastoma protein (pRB) is a critical substrate of activated G1-phase cyclin/CDK complexes. pRB is sequentially phosphorylated by cyclin D1/CDK4,6 and cyclin E/CDK2 during G1 progression and functions as either a transcriptional repressor or activator depending upon its phosphorylation state and co-associated proteins [3]. When hypo-phosphorylated, pRB binds to and represses members of the E2F-family of transcription factors [3]. The E2F family mediates transcription of genes required for DNA synthesis, thus the binding of hypo-phosphorylated pRB to E2F arrests cells at the G1/S transition. During G1 progression, CDK-mediated hyper-phosphorylation of pRB results in the dissociation of pRB and E2F, and S-phase entry ensues (Fig. 1).

G2- and M-phase progression is regulated by cyclin B1/CDC2 activity. During the G2-phase of the cell cycle, inactive cyclin B1/CDC2 complexes accumulate as the Wee1 and Myt1 kinases mediate inhibitory phosphorylations on CDC2 [4]. Mitotic entry requires activation of cyclin B1/CDC2 complexes by the CDC25C phosphatase, which removes the inhibitory phosphorylations on CDC2 [4]. Mitotic exit occurs after ubiquitination and proteolytic degradation of cyclin B1 by the anaphase-promoting complex (APC) inactivates CDC2 [2] (Fig. 2).

CELL CYCLE CHECKPOINTS

Eukaryotic cells have developed control mechanisms that restrain cell cycle transitions in response to stress, these regulatory pathways are known as cell cycle checkpoints [5]. Cells can transiently arrest at cell cycle checkpoints to allow for the repair of cellular damage. Alternatively, checkpoint signaling may activate pathways leading to programmed cell death if irreparable damage occurs. Loss of checkpoint integrity can permit the propagation of DNA lesions that results in permanent genomic alterations [5]. Checkpoint pathways that regulate cell cycle progression are frequently disrupted in tumor cells, underscoring the importance of intact checkpoint signaling for maintenance of the genome.

G1/S Checkpoint

Inhibition of G1-phase cyclin/CDK complexes plays a key role in G1/S checkpoint function. CDKs are negatively regulated by a group of functionally related proteins called CDK inhibitors (CDKIs) that fall into two families: the INK4 inhibitors and the Cip/Kip inhibitors [1]. There are four INK4 family members: p16INK4A, p15INK4B, p19INK4D, and p18INK4C, and three Cip/Kip family members: p21Waf1/Cip1, p27Kip1, and p57Kip2. The INK4 family specifically inhibits CDK4 and CDK6 activity during G1, while the Cip/Kip family can inhibit CDK activity during all cell cycle phases [1]. Both CDKI families play important roles in the G1/S checkpoint (Fig. 1). After exposure of normal cells to genotoxic agents, the CDKI p21Waf1/Cip1 (p21) is transcriptionally activated by the tumor suppressor protein p53. The elevated p21 protein binds and inactivates cyclin E/CDK2 complexes resulting in pRB hypo-phosphorylation and cell cycle arrest at the G1/S transition [6]. p16INK4A (p16) mediates the p53-independent G1 arrest in response to DNA damage in a variety of cell types, presumably through abrogation of CDK4- and CDK6-mediated phosphorylation of pRB [7].

G2 Checkpoint

Genotoxic stress also triggers checkpoint pathways that initiate a G2 cell cycle arrest. After DNA damage, ATM- and ATR-dependent signaling induces a G2 cell cycle arrest through inhibition of CDC2 (Fig. 2). ATM activates the Chk2 kinase in cells exposed to ionizing radiation, while ATR signaling mediates Chk1 activation in cells treated with ultraviolet radiation [8]. Chk1 and Chk2 phosphorylate CDC25C, generating a consensus binding site for 14-3-3 proteins. The binding of 14-3-3 proteins to CDC25C results in nuclear export and cytoplasmic sequestration of the phosphatase, with subsequent G2 arrest due to CDC2 inhibition [8]. Recent studies indicate p53 is required to sustain DNA damage-induced G2 arrest in tumor cells [9,10]. p53 maintains the G2 checkpoint through transcriptional upregulation of 14-3-3σ which sequesters CDC2 in the cytoplasm [11,12], as well as transactivation of p21 which inhibits the cyclin B1/CDC2 complex [10,13] (Fig. 2).

Mitotic Spindle Checkpoint

The mitotic spindle checkpoint monitors spindle microtubule structure and chromosome attachments, delaying chromosome segregation during anaphase until defects in the mitotic spindle apparatus are corrected (Fig. 3). The kinetochore-associated Mad2, BubR1, Bub1, and Bub3 proteins are critical constituents of the spindle checkpoint pathway. Mad2 and BubR1 regulate mitotic progression by direct interaction with and inhibition of the APC machinery to prevent anaphase entry in the presence of mitotic spindle dysfunction [14]. Bub1 and Bub3 also mediate mitotic arrest after microtubule disruption, as cells lacking Bub1 or Bub3 fail to undergo a mitotic arrest when treated with spindle disrupting agents [14].

CELL CYCLE DYSREGULATION IN HUMAN CANCERS

Loss of cell cycle checkpoint function is a hallmark of human cancers. Alterations in

components of the cell cycle machinery and checkpoint signaling pathways occur in the majority of human tumors. These genetic modifications ultimately result in dysregulation of oncogenes and tumor suppressor genes, a finding that has important implications for the optimization of current therapeutic regimens, as well as the selection of novel cell cycle targets.

Alterations of Constitutive Cell Cycle Machinery

Cell cycle regulation by the tumor suppressor protein pRB plays an integral role in the prevention of human tumors, as oncogenic alterations in cyclins, CDKs, and other upstream regulators of pRB occur in a variety of human tumors. Normal pRB function is defective in many human cancers, including breast, retinoblastoma, osteosarcoma, and lung [3]. In tumors lacking direct pRB gene mutation, modifications in components of the signaling pathways that regulate pRB are frequently noted, such as cyclin D1 and cyclin E overexpression, CDK4 and CDK6 gene amplification, or deletion of the CDKI p16 [3]. For example, nearly 50% of invasive breast cancers have elevated cyclin D1 expression as compared to surrounding normal breast epithelium [15], while transgenic mice that overexpress human cyclin D1 or cyclin E specifically in mammary cells develop mammary adenocarcinomas [16,17]. Similarly, CDK4 and CDK6 gene amplification occur in sarcomas, gliomas, melanomas, and breast cancers [18].

Alterations of Checkpoint Signaling Proteins

Genetic alterations involving cell cycle checkpoint signaling molecules are also common in human tumors. p53 gene mutation is the most frequently observed genetic lesion in human tumors [6]. The importance of p53-dependent signaling in tumor suppression is underscored by the finding that germline mutations of the p53 gene result in Li-Fraumeni syndrome, a highly penetrant familial cancer syndrome associated with significantly increased rates of brain tumors, breast cancers, and sarcomas [19]. In human tumors that lack p53 gene mutation, p53 function may be disrupted by alterations in cellular proteins that modulate the expression, localization, and biochemical activity of

p53. For example, the Mdm2 protein binds p53 and targets it for ubiquitin-mediated degradation during normal cellular growth conditions [20]. In some tumors possessing wild-type p53 alleles, Mdm2 gene amplification occurs, resulting in Mdm2 protein overexpression and subsequent p53 inactivation [21].

Modifications in CDKI function are also commonly found in human tumors. The CDKI p27 plays a significant role in breast cancer etiology. Many human breast cancers have aberrant p27 protein expression and reduced p27 protein levels are correlated with more aggressive breast tumors [22,23]. Further, p27 haplo-insufficiency renders murine mammary epithelium more susceptible to oncogene-dependent transformation [24]. Decreased expression of the CDKI p57 has also been reported in human bladder cancers [25]. Likewise, deletion or inactivation through methylation of the CDKI genes *p15* and *p16* is linked to the pathogenesis of human melanomas, lymphomas, mesotheliomas, and pancreatic cancers [18].

Mutation in other components of DNA damage response pathways may also lead to enhanced tumorigenesis. For example, ATM mutations occur in ataxia telangiectasia, a familial disease associated with an elevated incidence of leukemias and lymphomas [26]. Further, individuals harboring a heterozygous germline mutation of ATM have a five-fold increased incidence of breast cancer [26]. Mutations of Chk2 and Chk1 also arise in human cancers. Heterozygous alteration of Chk2 occurs in a subset of individuals with Li-Fraumeni syndrome that lack p53 gene mutations [27]. In addition, Chk2 mutations have been reported in human lung cancer [28], while Chk1 mutations have been observed in human colon and endometrial cancers [29].

Disruption of the spindle checkpoint also mediates tumor progression. Defective Bub1 or Mad2 function has been linked to the pathogenesis of several human tumors. Bub1 mutations have been identified in human colon carcinoma cells [30], and Bub1 mutation facilitates the transformation

of cells lacking the breast cancer susceptibility gene, BRCA2 [31]. Recent studies by Michel *et al.* demonstrate that Mad2 haplo-insufficiency results in significantly elevated rates of lung tumor development in Mad2+/- mice as compared to age-matched wild-type mice [32].

THERAPEUTIC MANIPULATION OF CELL CYCLE CHECKPOINTS

Preclinical studies indicate that cells with defective checkpoint function are more vulnerable to select anticancer agents, thus research efforts are now focused on identifying compounds that disrupt cell cycle checkpoints. These investigations include: (i) the development of chemical inhibitors through structure-based rational drug design, (ii) the use of high throughput screening assays, and (iii) the manipulation of genetic-based screening technologies to identify novel anticancer therapies.

Chemical Approaches

Since CDK activity is often deregulated in tumors, compounds that inhibit CDK function may be effective anticancer agents. The chemical CDKI flavopiridol arrests cancer cells at the G1/S and G2/M transitions through inhibition of CDK2, CDK4, and CDC2 kinase activity [33]. Flavopiridol has potent antiproliferative activity against a variety of human cancer cell lines, and xenograft tumor studies in mice indicate that flavopiridol acts synergistically with other anticancer agents [33]. Flavopiridol produced favorable clinical responses in Phase I and Phase II studies of patients with renal, colorectal, gastric, lung, and esophageal carcinomas, and current clinical trials are evaluating flavopiridol in non-Hodgkin's lymphoma, as well as breast and prostate cancers [18]. The clinical success of flavopiridol and related chemical CDKIs has spawned further research efforts to design mechanism-based CDKIs through manipulation of the phosphorylation and cyclin-binding sites of CDK proteins.

Numerous studies indicate that abrogation of the DNA damage-induced G2 arrest in cancer

cells enhances chemotherapeutic response. Tumor cells treated with caffeine or pentoxifylline, compounds that disrupt the G2 checkpoint, are sensitized to ionizing radiation [34,35]. Caffeine inhibits the ATM and ATR kinases, preventing Chk2 and Chk1 activation [8]. The effectiveness of targeting Chk1 to ablate the G2 checkpoint is exemplified by the use of the Chk1 inhibitors UCN-01 (7-hydroxystaurosporine) and SB-2180708 (a staurosporine-related compound) to increase the cytotoxic effect of DNA-damaging agents in tumor cells [36,37] . Promising preclinical results showing *in vivo* tumor growth inhibition by pentoxifylline and UCN-01 have prompted clinical trials to evaluate the efficacy of these compounds against a variety of tumors [38-40].

While many anticancer drugs target kinases involved in checkpoint signaling pathways, others regulate checkpoint function through inhibition of histone deacetylases or proteasome-dependent ubiquitination. Histone deacetylase inhibitors alter chromatin structure and gene expression, producing a G2 arrest in normal human cells but causing mitotic catastrophe in tumor cells [41]. The histone deacetylase inhibitors FR901228 and MS-27-275 have potent *in vitro* [42] and *in vivo* [43] anticancer activity, and FR901228 has demonstrated efficacy against T-cell lymphoma in clinical trials [44]. The ubiquitin-proteasome pathway is also an attractive target for anticancer drug manipulation, as this pathway mediates the degradation of cyclins and CDKIs [2]. PS-341 is a proteasome-specific inhibitor found to have significant *in vitro* cytotoxicity against a variety of human tumor cell lines and has shown favorable patient responses in Phase II clinical trials for melanoma, lung cancer, and sarcomas [45].

Screens for new compounds

Various screening strategies have been employed to identify novel anticancer agents. In one such effort, the National Cancer Institute (NCI) evaluated the anticancer activity of 70,000 compounds against a panel of 60 human tumor cell lines [46]. Subsequent studies found that the p53

status of these tumor cell lines was a critical determinant of cellular sensitivity to chemotherapeutic agents, as cell lines with mutant p53 exhibited less growth inhibition than those with wild-type p53 [47]. Amundson *et al.* recently used the NCI cell lines to evaluate the basal expression levels of transcripts from genes in checkpoint pathways and correlated the data with the sensitivity of the cells to standard chemotherapy agents [48]. Similarly, cDNA microarray analyses have been used to study the gene expression profiles of these cell lines in response to standard chemotherapeutic drugs [49]. These latter studies will provide valuable insight to how the expression of specific genes correlates with drug sensitivity.

High-throughput screens have also been used to identify novel compounds that disrupt checkpoint function. In one study, breast cancer cells expressing mutant p53 were grown in microtiter plates, irradiated to induce G2 arrest, and then co-treated with the microtubule inhibitor nocodazole and extracts from marine invertebrates [50]. This assay identified isogranulatimide, a novel G2 inhibitor that has synergistic cytotoxicity in combination therapy with ionizing radiation [50]. A similar microtiter plate assay was recently used to screen 24,000 extracts from marine invertebrates and terrestrial plants for novel antimitotic agents, resulting in the identification of 8 novel chemicals with antimitotic activity [51]. In another study, isogenic human colorectal cancer cell lines differing only at the K-Ras locus were stably transfected with an expression vector for either a yellow fluorescent protein or a blue fluorescent protein [52]. These cell lines were then co-cultured in the presence of 30,000 compounds, allowing the identification of compounds with selective toxicity toward the mutant Ras genotype. This latter approach identified a novel cytidine nucleoside analog that displayed selective activity toward tumor xenografts containing mutant Ras [52].

Genetic approaches

Yeast offer an attractive model system to evaluate chemotherapeutic agents due to the

conservation between yeast and mammalian cell checkpoint processes, the availability of yeast genomic sequences [53], and the ease of yeast genetic manipulation. In a recent study, a panel of isogenic yeast strains containing defined mutations in cell cycle checkpoint pathways was used to screen anticancer drugs [54]. The yeast strains exhibited differential toxicity profiles for several chemotherapeutic agents and ionizing radiation, suggesting that DNA repair and cell cycle checkpoint mutations in individual tumors may influence the outcome of a particular chemotherapeutic regimen. Similarly, budding yeast were employed to genetically select peptide inhibitors to identify novel cellular pathways to target for anticancer drug discovery [55]. In this latter study, peptides were selected based on phenotypic analyses followed by the genetic dissection of candidate target pathways to identify putative targets of the inhibitors [55]. The analysis identified a novel component of the spindle checkpoint, Ydr517w, thus exemplifying the power of genetic approaches in identifying novel components of existing biological pathways that may be potential targets for drug discovery. Yeast genomics can also be combined with cDNA microarray analyses to examine genome-wide changes in gene expression after treatment with anticancer regimens [56]. For example, a recent study used this approach to generate a database of expression profiles of various *S. cerevisiae* cell cycle mutants treated with a panel of chemotherapeutic compounds and ionizing radiation [57]. Analysis of these profiles revealed novel components of cell cycle signaling pathways.

CONCLUSIONS

Identifying the molecular differences between cancer cells and normal cells is crucial to the continued development of anticancer agents that can preferentially eliminate cancer cells and minimize the toxicity to normal tissues. The information generated by the genomic and proteomic projects of prokaryotic and eukaryotic organisms will continue to reveal new cell cycle regulatory

molecules. As our knowledge of cell cycle checkpoints increases, novel signaling molecules that can be targeted for rational drug design will be identified, allowing mechanism-based approaches to cancer treatment that exploit the molecular defects in tumors. To fully realize this potential, we need to continue to develop the technology to precisely define the checkpoint defects in individual tumors and treatment regimens tailored to the unique tumor cell cycle phenotype.

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Fig. 1. The G1/S Transition. During G1 phase progression, there is activation of cyclin D1/CDK4 and cyclin E/CDK2 complexes which sequentially phosphorylate the transcription factor pRB. Hypo-phosphorylated pRB binds to E2F to inhibit S phase entry; however, once hyper-phosphorylated, pRB releases E2F, resulting in activation of genes required for S-phase entry. Members of the INK4A and Cip/Kip CDKI families (represented by p16 and p21, respectively) can inhibit the cyclin/CDK kinase complexes to mediate a G1/S cell cycle arrest.

Fig. 2. G2 Checkpoint Activation After Genotoxic Stress. In response to genotoxic stress, the ATM/ATR signaling pathway is activated leading to phosphorylation and activation of Chk1 and Chk2 kinases and subsequent phosphorylation of Cdc25C. Phosphorylated CDC25C is sequestered in the cytoplasm by 14-3-3 proteins, preventing cyclin B1/CDC2 activation and resulting in G2 arrest. Activated ATM/ATR also activate p53-dependent signaling that contributes to maintenance of the G2 arrest by upregulating the 14-3-3σ protein that sequesters CDC2 in the cytoplasm. p53 also induces transactivation of the CDKI p21, that binds to cyclin/CDK complexes to reduce pRB phosphorylation and thus prevent E2F from mediating synthesis of cyclin B1 and CDC2. p21 also directly binds and inhibits cyclin B1/CDC2 complexes to block mitotic entry.

Fig. 3. The Spindle Checkpoint. Improper chromosome alignment on the mitotic spindle, disruption of microtubule dynamics, or unattached kinetochores can activate the spindle checkpoint. Spindle checkpoint signaling is mediated by the Bub1, Bub3, BubR1, and Mad2 proteins that all localize to kinetochores. A. Intact spindle checkpoint signaling induces either metaphase arrest through inhibition of APC or induction of apoptosis. B. Defective spindle checkpoint function from either loss of Bub1- and Bub3-dependent signaling or abrogation of Mad2/BubR1-mediated APC inhibition can lead to aberrant mitotic exit and, in the absence of a functional G1/S checkpoint, the generation of aneuploid cells.

Figure 1

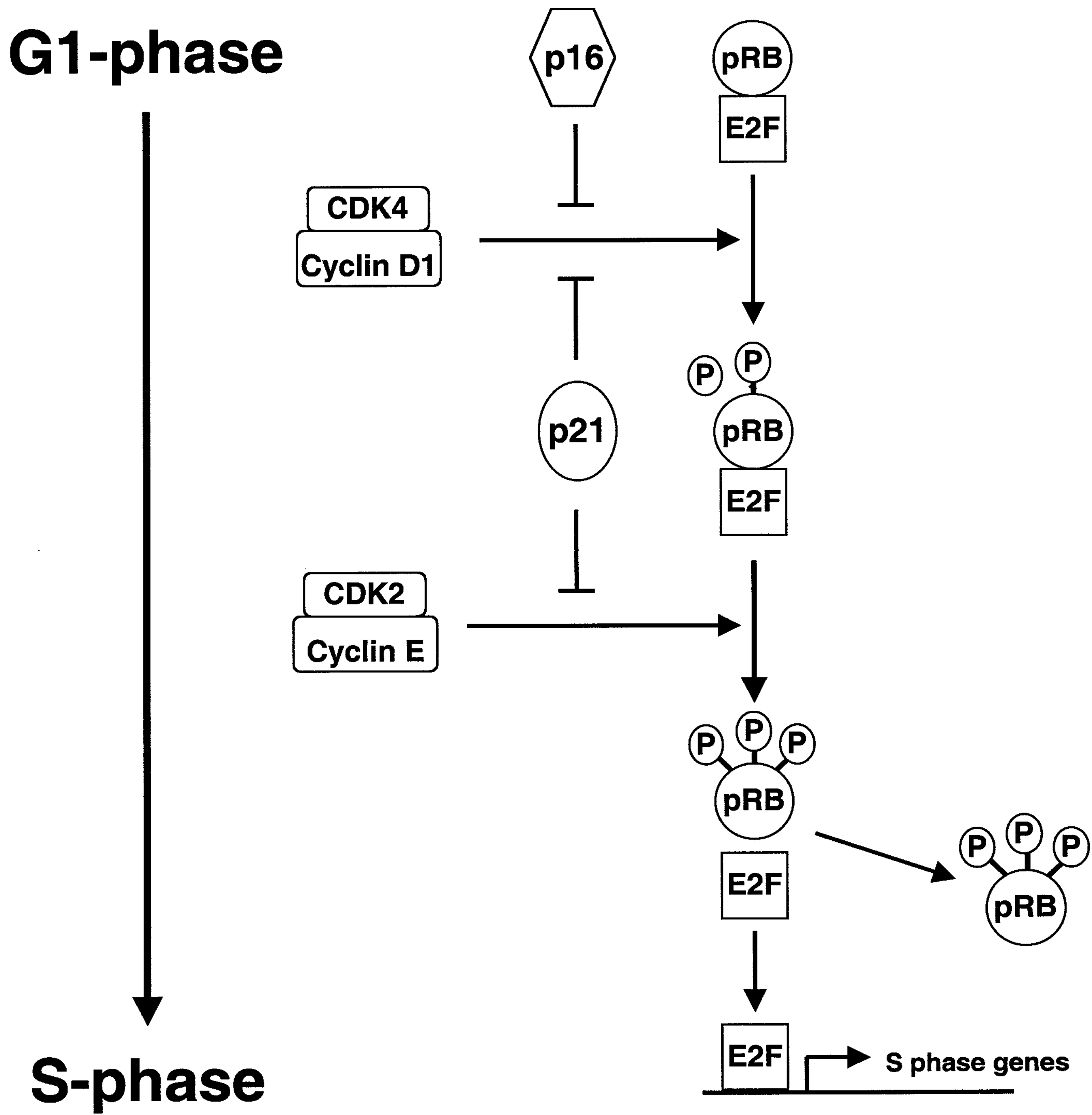


Figure 2

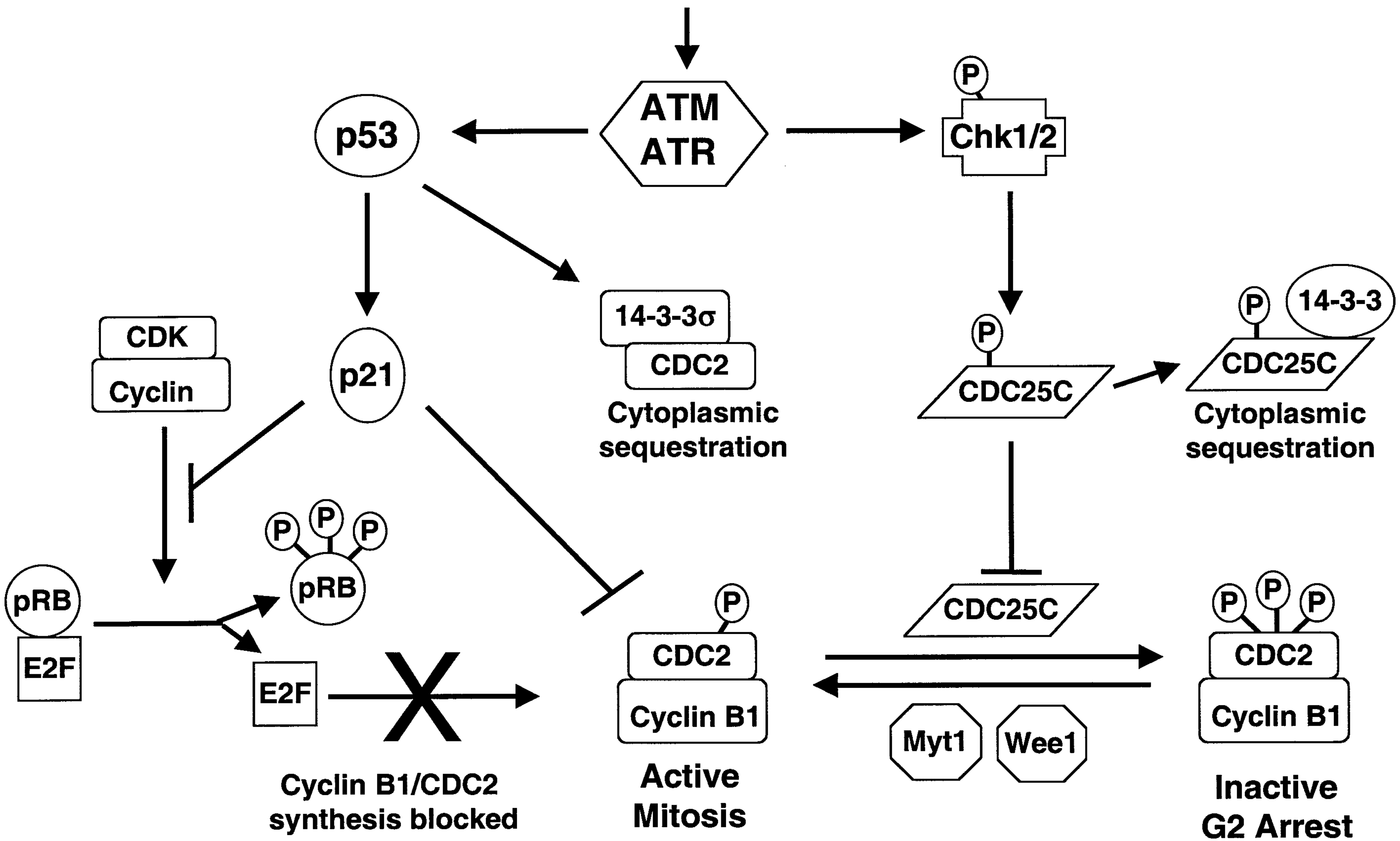
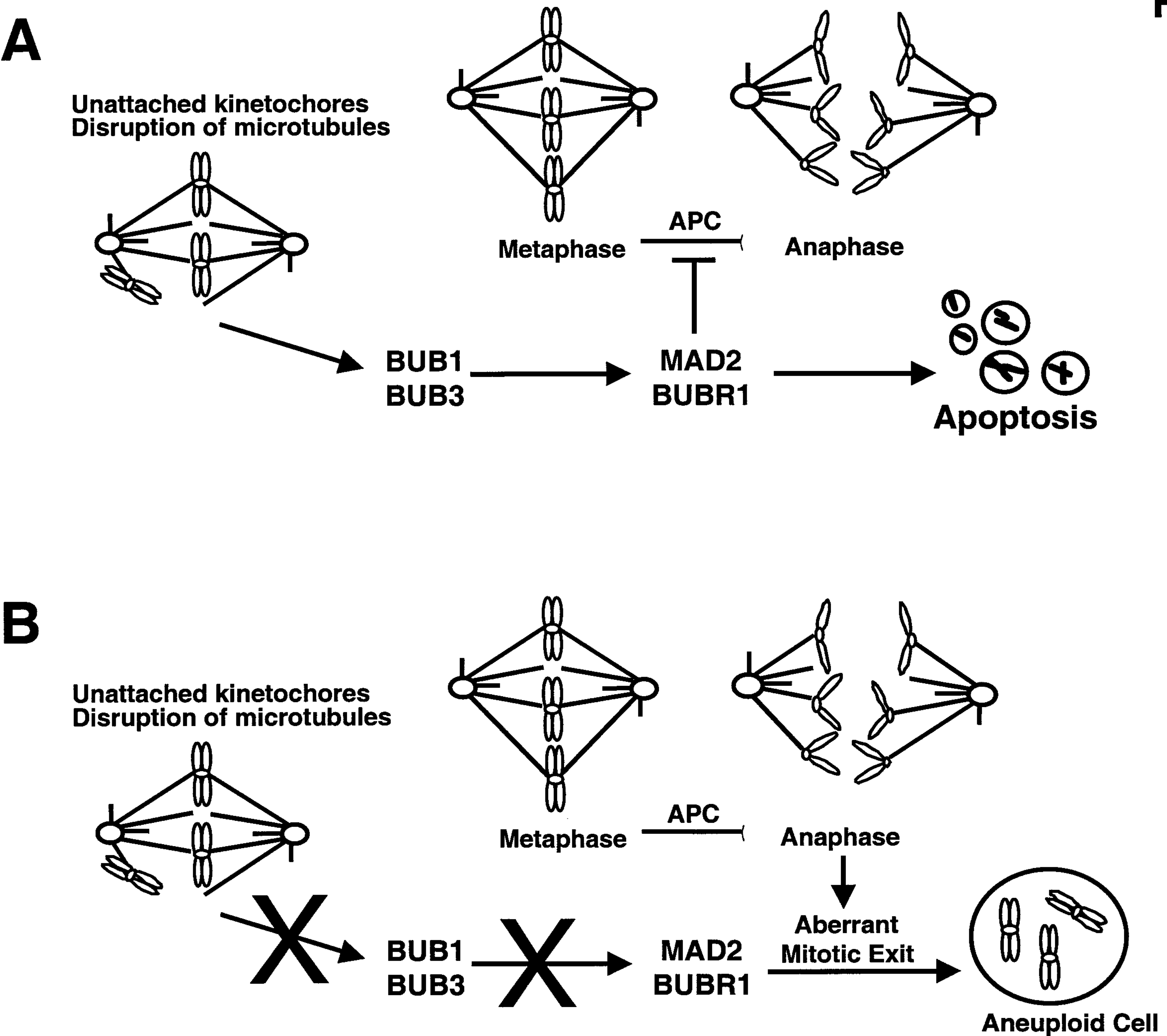


Figure 3



Biographical Sketches

Provide the following information for the key personnel listed on page 1 of the Detailed Cost Estimate form for the initial budget period.

NAME	POSITION TITLE
Eugenia Yazlovitskaya, Ph.D.	Research Assistant Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)

INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Kiev State University and Institute of Mol. Biol. & Genetics Kiev, Ukraine	M.S.	1984	Biochemistry
Palladin Institute of Biochemistry, Kiev, Ukraine	Ph.D.	1997	Biochemistry
University of Kansas Medical Center, Kansas City, KS	Postdoc	2001	Biochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds two pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED THREE PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INVESTIGATOR.

Professional Positions

1984-1992 Research Associate, Laboratory of Ecology, Central Botanical Garden, Ukrainian Academy of Science, Kiev, Ukraine

1992-1994 Research Assistant, Dept. of Microbiology, Immunology and Molecular Genetics, University of Kansas Medical Center, Kansas, USA

1994-1996. Research Assistant, Dept. of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas, USA

1996-1997 Research Associate, Dept. of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas, USA

1997-2001 Postdoctoral Research Associate, Dept. of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas, USA

2001-2002 Research Assistant Professor, Dept. of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas, USA

2002-pres. Research Assistant Professor, Dept. of Biochemistry, Vanderbilt University, Nashville, Tennessee, USA

Grants - Professional Activities

2000-2001 KUMC Postdoctoral Training Grant in Biomedical Research, Kansas City, KS

2001 American Association for Cancer Research Pathobiology of Cancer Workshop Scholarship, Keystone, CO

Professional Socities

American Association for Cancer Research (since 1997)

American Association for Cancer Research - Women in Cancer Research (since 1997)

Publications

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7. E.M.Yazlovitskaya, J.C.Pelling and D.L.Persons. (1999). Association of apoptosis with the inhibition of extracellular signal-regulated protein kinase activity in the tumor necrosis factor _-resistant ovarian carcinoma cell line UCI 101. Molecular Carcinogenesis, 25: 14-20.

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10. W. Cui, E.M.Yazlovitskaya, M.S.Mayo, J.C.Pelling and D.L.Persons. (2000). Cisplatin-induced response of c-jun-N-terminal kinase 1 and extracellular signal regulated protein kinase 1 and 2 in a series of cisplatin-resistant ovarian carcinoma cell lines. Molecular Carcinogenesis, 29: 219-228.

11. E.M.Yazlovitskaya, R.D.DeHaan and D.L.Persons. (2001). Prolonged wild type p53 protein accumulation and cisplatin resistance. Biochemical and Biophysical Research Communications, 283: 732-737.

12. R.D.DeHaan, E.M.Yazlovitskaya and D.L.Persons.(2001). Regulation of p53 target gene expression by cisplatin-induced extracellular signal-regulated kinase. Cancer Chemotherapy and Pharmacology, 48: 383-388.

13. K.SantaCruz, E.M.Yazlovitskaya, J.Collins, J.Johnson and C.DeCarli. Regional NAD(P)H: quinone oxidoreductase activity in Alzheimer's disease. Submitted to Annals of Neurology.

14. E.M.Yazlovitskaya and D.L.Persons. Cisplatin-induced activation of the ATR dependent pathway in A2780 ovarian carcinoma cells. Submitted to Biochemical and Biophysical Research Communications.

15. E.M.Yazlovitskaya, W.Nudson, J.Neat and D.L.Persons. Regulation of Mdm2 protein function by extracellular signal-regulated protein kinase (ERK1/2) during cisplatin treatment. Submitted.

Abstracts:

1. G.Melnykovych, P.A.Voziyan, J.S.Haug, Ch.M.Goldner, E.M.Yazlovitskaya (1993). Effect of farnesol on growth and phospholipid biosynthesis in CEM-C1 line of human acute lymphoblastic leukemia. Abstracts of the Satellite Meeting to the 12th International Symposium on Glukokonjugates. Zacopane, Poland.

2. P.A.Voziyan, J.S.Haug, E.M.Yazlovitskaya, I.Adany and G.Melnykovych (1994). Farnesol induces apoptosis in cultured cells: comparison of neoplastic and non neoplastic cells. Proceedings of 85th Annual AACR Meeting. San Francisco, CA.

3. E.M.Yazlovitskaya and G.Melnykovych (1994). Toxic effect of Farnesol on cell viability is preceded by inhibition of protein kinase C (PKC) activity: differences between neoplastic HeLaS3K cells and non-neoplastic CF-3 cells. Abstracts of 1994 Annual AICR Research Conference. Washington, DC.

4. G. Melnykovych, P.A. Voziyan, J.S. Haug, E.M. Yazlovitskaya (1995). Mechanism of farnesol-induced apoptotic cell death may involve PKC-dependent signal transduction pathway. Proceedings of 86th Annual AACR Meeting. Toronto, Ontario, Canada.

5. S.A. Li, E.M. Yazlovitskaya, J.J. Li (1996). Over expression of cathepsin D during estrogen-induced tumorigenesis in the hamster: Possible role in estrogen-mediated tubular cell damage. Proceedings of 87th Annual AACR Meeting. Washington, D.C.

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10. D.L. Persons, E.M. Yazlovitskaya and J.C. Pelling (2000). Extracellular signal regulated protein kinase (ERK) activity and regulation of p53 protein accumulation during exposure to cisplatin. Proceedings of 91st Annual AACR Meeting, San Francisco, CA.

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12. K.S. SantaCruz, E. Yazlovitskaya, J. Collins, A.B. Acharya, M. Haque, J. Johnson, F. Samson, C. DeCarli (2000). NADPH: Quinone oxidoreductase is increased in hippocampal neurons and cortical astrocytes in Alzheimer's disease. Free Radical Biology and Medicine, 29, S1: S128.

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