

AD _____

Award Number: DAMD17-00-1-0295

TITLE: Role of DNA-Dependent Protein Kinase in Breast Cancer
Development/Progression

PRINCIPAL INVESTIGATOR: Suk Hee Lee, Ph.D.

CONTRACTING ORGANIZATION: Indiana University
Indianapolis, Indiana 46202-5167

REPORT DATE: July 2001

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 2001	3. REPORT TYPE AND DATES COVERED Annual (15 Jul 00 - 14 Jun 01)	
4. TITLE AND SUBTITLE Role of DNA-Dependent Protein Kinase in Breast Cancer Development/Progression			5. FUNDING NUMBERS DAMD17-00-1-0295	
6. AUTHOR(S) Suk Hee Lee, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Indiana University Indianapolis, Indiana 46202-5167 E-Mail: slee@iupui.edu			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. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			20011127 027	
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) DNA-dependent protein kinase (DNA-PK) is a nuclear serine/threonine protein kinase involved in various DNA metabolism and damage signaling pathways. DNA damage activates DNA-PK, which in turn phosphorylates a number of key proteins involved in replication, repair, and transcription. Accordingly, DNA-PK has long been suspected as a factor involved in sensing and transmitting DNA damage signals to the downstream target, which eventually contributes to the genomic stability and prevention of cancer. The overall goal of this proposal is to explore the role of DNA-PK in the development and progression of breast cancer. Since DNA-PK is a DNA repair factor as well as involved in damage signaling pathway, levels of DNA-PK activity among breast cancer would contribute to their drug resistance and also provide the basis for selection of patients for treatment with chemotherapy drugs. From the first year of the study we concluded that a peptide-based inhibitor preventing DNA-PKcs from forming a complex with Ku70/Ku80 significantly lowered DNA-PK activity. Furthermore, treatment of these breast cancer cells with target peptide significantly lowered the cell growth only in the presence of ionizing radiation, indicating that the peptide-based inhibitor exhibited a positive effect of on lowering drug resistance by specifically targeting DNA-PK <i>in vivo</i> . This result also validates a physiologic role for DNA-PK in chemotherapy drug resistance of breast cancers.				
14. SUBJECT TERMS Damaged-induced cell cycle checkpoint, DNA repair, DNA replication, S-phase arrest			15. NUMBER OF PAGES 8	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusions.....	8
References.....	8
Appendices.....	8

INTRODUCTION:

The overall goal of this proposal is to explore the role of DNA-dependent protein kinase (DNA-PK) in the development and progression of breast cancer. DNA-PK is a nuclear serine/threonine protein kinase involved in various DNA metabolism and damage signaling pathways. DNA damage activates DNA-PK, which in turn phosphorylates a number of key proteins involved in replication, repair, and transcription. Accordingly, DNA-PK has long been suspected as a factor involved in sensing and transmitting DNA damage signals to the downstream target, which eventually contributes to the genomic stability and prevention of cancer. To see a correlation between DNA-PK activity and different stages of cancer, DNA-PK activity levels of various stages of breast cancer will be analyzed. Outcome of this study will provide us the information as to whether DNA-PK can be used as a prognostic measure of breast cancer progression. We will also analyze breast cancer cells grown in culture and grown as tumor grafts in mice to see whether DNA-PK plays a role in chemotherapy resistance. A strong correlation between DNA-PK activity and drug resistance of breast cancers would provide the basis for selection of patients for treatment with chemotherapy drugs. Furthermore, the information regarding the role of DNA-PK in drug resistance would be very useful for drug discovery aimed at increasing the sensitivity of tumors to chemotherapy.

BODY:

Task 1. To characterize the relationship between DNA-PK and breast cancer development/progression (months 1-36)

In order to assess DNA-PK activity and its expression in various stages of breast cancer cells, we are in the process of obtaining tissue samples from the Indiana University Cancer Center (IUCC) Tumor Bank and the Gynecologic Oncology Group (GOG) Tumor Bank. We have had some difficulty in establishing number of specimens for different stages of breast cancer by the FIGO (Federation of International Gynecologic Organization) guideline however now we are in the position to collect enough number of specimens for our study. Once established them all, tissue samples will be grown in tissue culture dish (25 x 150 mm) and cell extracts will be prepared for measurement of DNA-PK activity. Also, molecular analysis of the alteration of DNA-PK activity in breast cancers will follow to evaluate the relationship between DNA-PK activity and the degree status of breast cancer.

Task 2. To determine whether DNA-PK plays a role in DNA repair and/or the chemotherapy drug resistance among breast cancers (months 1-36)

DNA-PK activity and chemotherapy drug resistance of breast cancer cells (months 6-24):

We initially proposed to determine whether altered DNA-PK activity in breast cancers is associated with chemotherapy drug. In order to see the relationship between DNA-PK and drug resistance of breast cancer cells, we prepared a peptide-based inhibitor that binds to the C-terminus of Ku80 (Gell & Jackson, 1999) and competitively inhibits DNA-PKcs from binding to Ku70/Ku80.

Peptide-based inhibitor contains the hydrophobic region localization sequence, so-called membrane-translocating carrier, which not only facilitates secretion of proteins, but also is important for importing synthetic peptides into the cell (Lin YZ *et al*, 1995). This localization peptide is capable of carrying a functional domain such as nuclear localization signal (NLS) (Boulikas T, 1994). We therefore synthesized a 38-residue peptide (HNI-38) comprising the signal peptide sequence (AAVALLPAVLLALLAP) and NLS (VQRKRQKLM) followed by a tyrosine (Y) residue and 12-residue of peptide inhibitor sequence (EGGDVDDLLDMI) representing the C-terminus of Ku80 (amino

acids #721-732). In addition to HNI-38, a control peptide (HN-26) was synthesized to see whether peptide inhibitor functions properly *in vivo* (see Figure 1). The tyrosine residue was used for ^{125}I -labeling to determine the import efficiency of synthetic peptides into the cells (and nuclei) (Lin YZ *et al*, 1995).

HNI-38: AAVALLPAVLLALLAPVQRKRQKLMY—**████████████████████**
 HN-26: AAVALLPAVLLALLAPVQRKRQKLMY
 HI-29: AAVALLPAVLLALLAPY—**████████████████████**
 NI-22: VQRKRQKLMY—**████████████████████**

Figure 1. Sequences of synthetic peptide-based inhibitor and control peptides (single-letter amino acid code). The membrane-translocating hydrophobic signal sequence is underlined and the nuclear localization sequence is shown in **bold face**. Twelve residue of peptide inhibitor region is indicated as bar (████) at the C-terminus. The tyrosine residue (Y) is included for ^{125}I -labeling to determine the import efficiency of the peptide into the cells.

We tested these peptides for their effects on DNA-PK kinase activity *in vitro* using a specific substrate peptide (EPPLSQEAFADLWKK) (Lees-Miller *et al*, 1990). The target peptide (HNI-38) effectively inhibited DNA-PK activity under the conditions where the control peptide (HN-26) had no effect, indicating that HNI-38 prevented DNA-PKcs from forming a complex with Ku70/Ku80 (Figure 2).

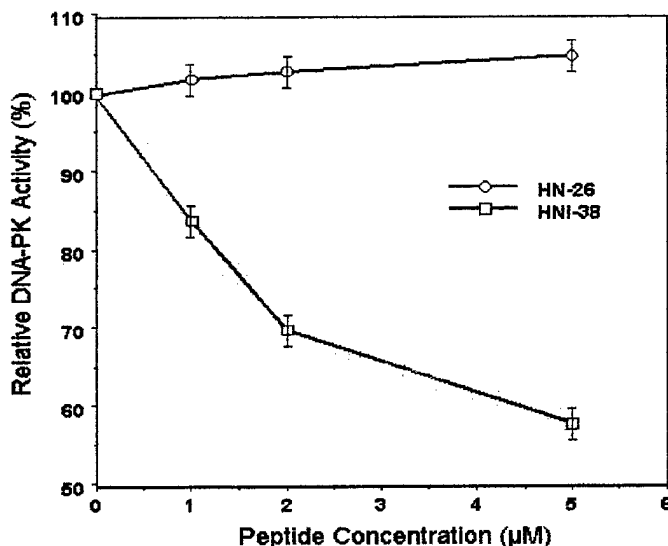


Figure 2. Effect of HNI-38 on DNA-PK activity. Reaction mixtures (20 µl) contained kinase buffer [20 mM HEPES (pH7.5), 1 mM EGTA, 1 mM EDTA, 1 mM DTT, 10 mM MgCl₂, 7 mM MnCl₂, 5 mM NaF, 1 mM Na₃VO₄, and 50 µM of γ - ^{32}P -ATP, 150 µM of specific substrate peptide (EPPLSQEAFADLWKK), 0.4 µg of calf-thymus activated DNA, 50 ng of purified DNA-PK, and the indicated amount of peptide. After 30 min incubation at 30°C, the reaction mixtures were stopped by 30% acetic acids. 5µl of reaction mixtures was spotted onto a P81 strip and, after extensive washing, radioactivity was measured. DNA-PK activity was shown as pmol of ^{32}P -ATP transferred to the substrate peptide.

A previous study indicates that DNA-PK mutant cells exhibit sensitivity to irradiation and cisplatin treatment (Britten *et al*, 1999; Frit *et al*, 1999). Also, studies with drug-resistant and sensitive cells indicate that higher levels of DNA-PK expression lead to drug-resistant cells, whereas the low DNA-PK activity was associated with cells with drug-sensitive phenotype (Shen *et al*, 1997). Since the target peptide effectively interferes with DNA-PK activity, we attempted to analyze whether alteration of DNA-PK activity induced by treatment with peptide-based inhibitor affected drug resistance of breast cancer cells (Figs. 3-5).

Two different breast cancer cells with different DNA-PK activity (MDA-231 and MDA-435) were analyzed for their resistance to ionizing radiation using standard colony count cell survival assay. Treatment of these breast cancer cells with target peptide significantly lowered the cell growth only in the presence of ionizing radiation (Figs. 3 & 4), indicating that the peptide-based inhibitor exhibited a positive effect of on lowering drug resistance by specifically targeting DNA-PK *in vivo*. This result also validates a physiologic role for DNA-PK in chemotherapy drug resistance of breast cancers.

Fig. 3. Effect of DNA-PK inhibitory peptide (HNI-38) on cell survival of breast cancer cell, MDA-231. Average 200 cells were plated in T-25 flasks and, after 24 hr incubation, cells were treated with γ -irradiation (0 Gy; upper panel, 2 Gy; lower panel). Following incubation at 37°C CO₂ incubator for 4 days, cells were stained with crystal violet and colonies (>50 cells) were counted under the light microscope.

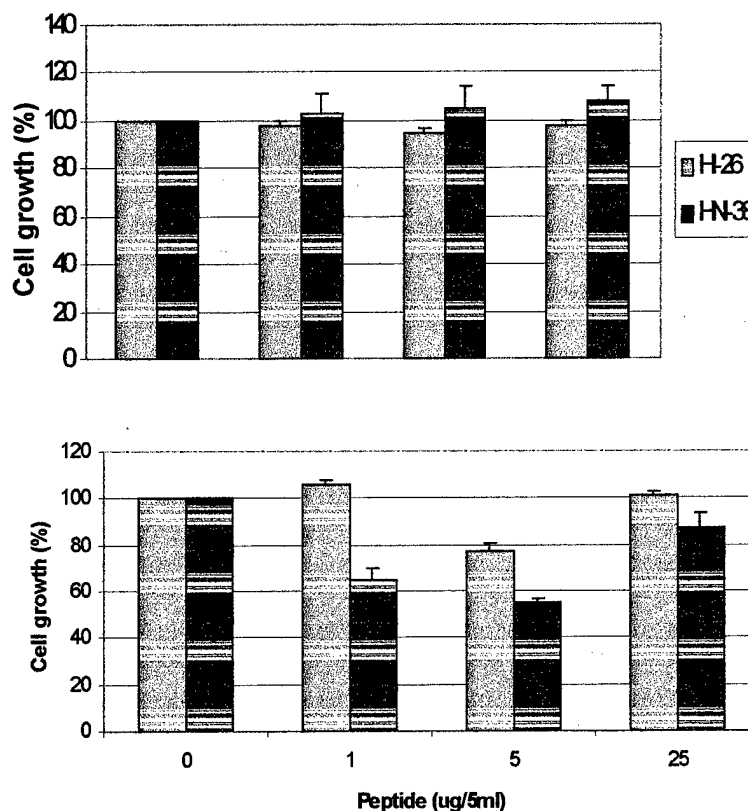
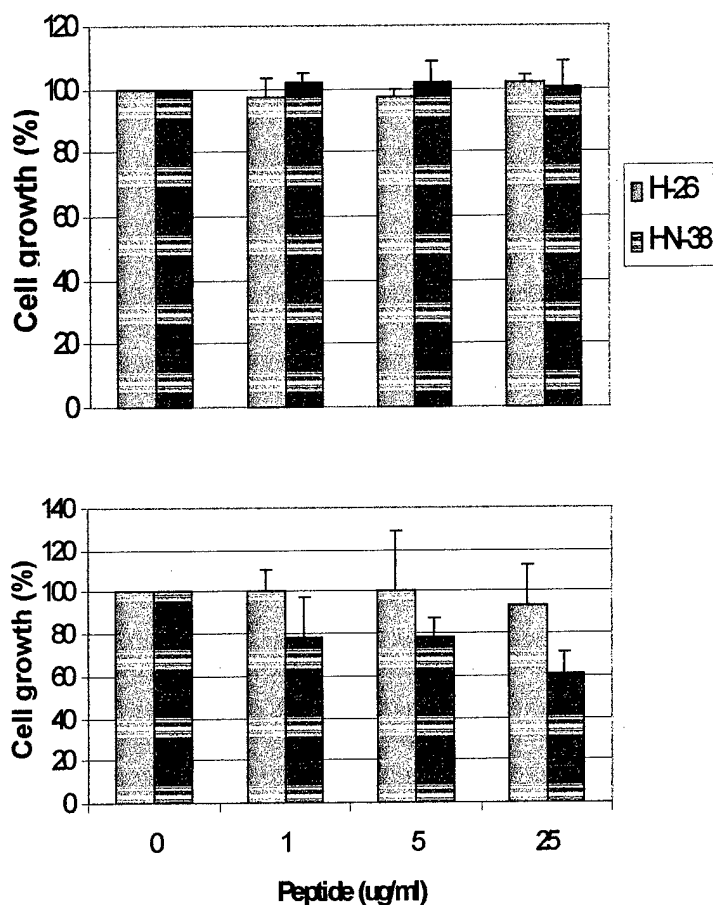


Fig. 4. Effect of DNA-PK inhibitory peptide (HNI-38) on cell survival of breast cancer cell, MDA-435. Cells were treated with γ -irradiation (0 Gy; upper panel, 2 Gy; lower panel) as described in Fig. 3. Following incubation at 37°C CO₂ incubator for 4 days, cells were stained with crystal violet and colonies (>50 cells) were counted under the light microscope.



KEY RESEARCH ACCOMPLISHMENTS:

1. Demonstration of the relationship between DNA-PK activity and drug resistance of breast cancer cells
2. Design and the synthesis of a peptide-based inhibitor of DNA-PK that lowers the growth of breast cancer cells following radiation treatment

REPORTABLE OUTCOMES:

1. Park, S-J, Kim, C-H, and Lee, S-H (2001) Sensitization of breast cancer cells by a targeted inhibition of DNA-dependent protein kinase (manuscript in preparation).
2. Invited presentation at the annual meeting for The Amelia Project (The Catherine Peachey Fund for Breast Cancer Research), Indianapolis, IN, February 2001.

CONCLUSIONS:

The overall goal of this proposal is to explore the role of DNA-PK in the development and progression of breast cancer. Since DNA-PK is a DNA repair factor as well as involved in damage signaling pathway, levels of DNA-PK activity among breast cancer would contribute to their drug resistance and also provide the basis for selection of patients for treatment with chemotherapy drugs.

From the first year of the study we concluded that a peptide-based inhibitor preventing DNA-PKcs from forming a complex with Ku70/Ku80 significantly lowered DNA-PK activity. Furthermore, treatment of these breast cancer cells with target peptide significantly lowered the cell growth only in the presence of ionizing radiation, indicating that the peptide-based inhibitor exhibited a positive effect of on lowering drug resistance by specifically targeting DNA-PK *in vivo*. This result also validates a physiologic role for DNA-PK in chemotherapy drug resistance of breast cancers.

REFERENCES:

- Lin, YZ, Yao SY, Veach RA, Torgerson TR, Hawiger J (1995) Inhibition of nuclear translocation of nuclear transcription factor NF-KB by a synthetic peptide containing cell membrane-permeable motif and nuclear localization sequence J. Biol. Chem., 270(24), 14255-14258.
- Boulikas T (1994) Nuclear import of protein kinases and cyclins. J Cell Biochem., 55(1), 32-58.
- Gell D, Jackson SP. (1999) Mapping of protein-protein interactions within the DNA-dependent protein kinase complex. Nucleic Acids Res. 27(17), 3494-3502.
- Lees-Miller, S.P., Chen, Y.-R. and Anderson, C.W. (1990) Human cells contain a DNA-activated protein kinase that phosphorylates simian virus 40 T antigen, mouse p53, and the human Ku autoantigen. Mol. Cell. Biol., 10: 6472-6481.
- Britten, RA, Kuny, S, Perdue, S (1999) Modification of non-conservative double-strand break (DSB) rejoining activity after the induction of cisplatin resistance in human tumour cells. Br. J. Cancer, 79, 843-849.
- Frit, P, Canitrot, Y, Muller, C, Foray, N, Calsou, P, Marangoni, E, Bourhis, J, Salles, B (1999) Cross-resistance to ionizing radiation in a murine leukemic cell line resistant to cis-dichlorodiammineplatinum(II): role of Ku autoantigen. Mol. Pharmacol., 56(1), 141-146.
- Shen, H., Kauvar, L. and Tew, K.D. (1997) Importance of glutathione and associated enzymes in drug response. Oncol. Res., 9(6): 295-302

APPENDICES:

None