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Award Number: W81XWH-04-1-0764

TITLE: Identification of a Potent Apoptotic Peptide Produced by Fibroblasts: Studies Toward the Design of a Novel Agent for Breast Cancer Therapy

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REPORT DATE: September 2005

TYPE OF REPORT: Annual

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 *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-09-2005	Annual	6 Aug 2004 – 5 Aug 2005

4. TITLE AND SUBTITLE
Identification of a Potent Apoptotic Peptide Produced by Fibroblasts: Studies Toward the Design of a Novel Agent for Breast Cancer Therapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0764

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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E-mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Albany Medical College
Albany, NY 12208

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
We previously showed that constitutive activation of the platelet-derived growth factor beta receptor (PDGFR) in mortal human fibroblasts (HDFs) by the bovine papillomavirus E5 or the v-Sis oncoprotein induces partial transformation of these cells. However, two weeks after they reach their peak density E5- and v-Sis-expressing HDFs secrete a small, hydrophilic peptide that induces massive apoptosis in an autocrine manner. Specifically, this peptide induces a type of caspase-independent, Bcl-2-resistant apoptosis by promoting mitochondrial dysfunction, which results in the release of the apoptotic mitochondrial protein AIF into the cytosol and its subsequent translocation to the nucleus. We hypothesize that as a negative feedback response to sustained PDGFR signaling, HDFs release a small, hydrophilic peptide that induces apoptosis by activating or sensitizing pro-apoptotic Bcl-2-related proteins such as Bax, which in turn promote mitochondrial dysfunction. The primary goal of this project is to identify this apoptotic peptide produced by partially transformed HDFs. To date, we have further characterized this peptide and are in the process of designing a scheme for purification of the peptide by ion-exchange chromatography. Furthermore, we obtained evidence that the peptide disrupts the redox homeostasis in the cells. Since this peptide can induce apoptosis of a number of different tumor cell lines including MCF-7 and MDA human breast carcinoma cells, once identified it could serve as the forerunner of a novel anti-breast cancer agent.

15. SUBJECT TERMS
Apoptosis, peptide, tyrosine kinase

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	5	USAMRMC

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INTRODUCTION:

We have been investigating sustained activation of growth factor receptor tyrosine kinases in mortal human fibroblasts (HDFs). Constitutive activation of the platelet-derived growth factor beta receptor (PDGFR) by the bovine papillomavirus E5 or the v-Sis oncoprotein induces partial transformation of HDFs. However, two weeks after they reach their peak density HDFs partially transformed by E5 or v-Sis undergo massive apoptosis. Our evidence suggests that these cells secrete a small, protease-sensitive, hydrophilic factor that induces apoptosis in an autocrine manner. Specifically, this factor induces a type of caspase-independent, Bcl-2-resistant apoptosis by promoting mitochondrial dysfunction, which results in the release of the apoptotic mitochondrial protein AIF into the cytosol and its subsequent translocation to the nucleus. We hypothesize that as a negative feedback response to sustained PDGFR signaling, HDFs release a small, hydrophilic peptide that induces apoptosis by activating or sensitizing pro-apoptotic Bcl-2-related proteins such as Bax, which in turn promote mitochondrial dysfunction. The primary goal of this project is to identify this apoptotic peptide produced by partially transformed HDFs. Since this peptide can induce apoptosis of a number of different tumor cell lines including MCF-7 and MDA human breast carcinoma cells, once identified it could serve as the forerunner of a novel anti-breast cancer agent.

PROGRESS REPORT:

To date we have made progress on Task 1, to purify the peptide using a conventional biochemical approach. We have attempted to purify the peptide by various chromatographic methods. We can successfully isolate the apoptotic activity in the low molecular weight medium fraction using a centrifugal filtration devise with a molecular weight cut off size of 3 kilodaltons. Also, the apoptotic peptide does not bind to a reverse phase column, indicating that it is hydrophilic. However, we were unable to isolate the peptide using a resin that specifically binds hydrophilic compounds. Currently we are attempting to purify the peptide from the low molecular weight fraction of the apoptotic medium by ion exchange chromatography. Preliminary results indicate that the peptide does not bind to a cationic column, suggesting that the peptide may have an overall negative charge. Therefore, we will attempt to purify the peptide using an anionic column.

In order to design an alternative, feasible method for purification of the peptide we have further characterized it. First, we found that the peptide is sensitive to a pH change. Specifically, it appears to be active at a slightly higher than physiologic pH (i.e., it is active at pH 7.5-8.0 but not at pH 7). Second, a low concentration of hydrogen peroxide, which is not apoptotic by itself, increases the apoptotic activity of the peptide. Third, we found that treatment of the peptide with iodoacetamide decreases its apoptotic activity. Taken together, these observations suggest that the apoptotic peptide contains one or more cysteine residues that must be oxidized to form either an interchain or intrachain disulfide bond in order to for the peptide to be apoptotic. Therefore, we can use this property to design an alternative purification strategy for the apoptotic peptide. Specifically, we will attempt to biotinylate the peptide using a regent that reversibly couples biotin to a sulfydryl group and then purify the biotinylated peptide by affinity chromatography using avidin agarose.

We also observed that the apoptotic peptide is not produced or activated when the E5-expressing HDFs are cultured in DMEM rather than MEM-alpha medium. Therefore, we are currently attempting to identify components that are present in MEM-alpha and not in DMEM that enable production/activation of the peptide. This may provide further clues regarding the identity of the peptide and/or how it is produced.

Finally, we obtained evidence regarding the mechanism by which this apoptotic peptide works. Specifically, we have data to suggest that the peptide induces oxidative stress in HDFs, as a number of proteins involved in redox homeostasis appear to be upregulated in response to the peptide. These include manganese superoxide dismutase, thioredoxin, thioredoxin peroxidase, peroxiredoxin, glutathione S transferase, and the HSP 70 family of heat shock proteins. Thus, it is possible that the peptide induces apoptosis by triggering the production of hydrogen peroxide.

Summary of key Accomplishments:

- Discovered that the peptide may have an overall negative charge and therefore may be isolated by anion exchange chromatography.
- Further characterized the peptide as containing a cysteine residue, which would enable an alternative means of purifying the peptide.
- Observed that there may be certain medium components that allow for production or activation of the apoptotic peptide.
- Obtained evidence that the peptide triggers oxidative stress.

CONCLUSIONS:

Although we have been unable to purify the apoptotic peptide, so far we have identified several additional properties of the peptide which should enable us to more readily purify it in the future. Based on the new data obtained in the past year, we speculate that the apoptotic peptide may contain a disulfide bond, which acts as an oxidizing moiety, and thereby induces apoptosis by triggering oxidative stress in the cells.