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**13. ABSTRACT** *(Maximum 200 Words)*

E2A gene products (E47 and E12) are potent transcription factors containing the basic helix-loop-helix (bHLH) domain for DNA binding and dimerization. E2A proteins are thought to control cell growth and differentiation. E2A is also implicated to have a role as a tumor suppressor. E2A function can be inhibited by a group of inhibitors called Id. Thus, overexpression of Id proteins could diminish the function of E2A as a tumor suppressor, and potentially cause breast cancer. To test this hypothesis, we have generated transgenic mice expressing Id-1 in mammary epithelial cells, but have not detected any tumor formation. On the other hand, we have found Id-1 overexpression in a large fraction of archived human breast cancer samples. The significance of Id-1 overexpression in human breast cancer remains to be understood.

Additionally, we have shown that E2A proteins in primary mammary epithelial cells and in the MDA-MB-231 breast cancer cell line are degraded through a proteasome mediated pathway. Furthermore, activation of the Notch signaling pathway has been found to accelerate the degradation of E2A proteins. The molecular mechanism underlying Notch-mediated degradation has been investigated. It is therefore possible that activation of Notch signaling pathway associated with mammary tumor formation may involve the elimination of E2A proteins, the putative tumor suppressors.

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## TABLE OF CONTENTS

## INTRODUCTION

The central hypothesis of this proposed research was that the basic helix-loop-helix transcription factors represented by the products of the E2A gene might act as tumor suppressors in mammary epithelial cells as they do in lymphoid cells. Therefore, overexpression of their inhibitors, Id proteins, in experimental animal models or in humans would lead to tumorigenesis.

The E2A gene is ubiquitously expressed and encodes two alternatively spliced products, E12 and E47, which are potent transcription factors containing the basic helix-loop-helix (bHLH) domain for DNA binding and dimerization (Murre et al., 1989). E12 and E47 form homodimers or heterodimers with other tissue-specifically expressed bHLH transcription factors to bind to a consensus sequence called E box. These bHLH proteins have been shown to play crucial roles in the differentiation of a variety of cell types such as the lymphocytes, muscle cells, pancreatic cells, and neuronal cells (Zhuang et al., 1994; Bain et al., 1994; Weintraub. et al., 1991; Naya et al., 1995; Lee et al., 1995). In addition to dimerizing with the bHLH proteins and binding to DNA, E12 and E47 can also form heterodimers with the Id proteins (Id1-4), which has the HLH domain for dimerization but not the basic region for DNA binding. Therefore, E12 and E47 can be sequestered into inactive complexes and their function as transcription factors can be inhibited. Indeed, it has been shown that overexpression of Id proteins blocks the differentiation of many cell lineages including the B and T lymphoid, muscle, neuronal, adipose and mammary epithelial cells (Sun, 1994; Kim et al., 1999; Jen et al., 1992; Moldes et al., 1997; Desprez et al., 1995).

Apart from the differentiation function of E2A gene products, they have also been implicated to have a role as tumor suppressors. In NIH 3T3 fibroblasts, overexpression of E47 or incubation with Id antisense oligonucleotides arrests cell cycle at the G1 to S phase transition (Peverali, et al., 1994; Hara et al., 1994; Hara et al., 1994). We have shown that E2A can activate transcription of the gene encoding cyclin dependent kinase inhibitor, $p21^{CIP}$, through the E box sequences located in the promoter region of the p21 gene (Prabhu et al., 1997). In mice, disruption of the E2A gene (Bain et al., 1996; Yan et al., 1997) or overexpression of the Id-1 inhibitor in the T cell lineage (Kim et al., 1999) results in the development of T cell lymphoma at very high frequencies, thus suggesting a tumor suppressor function for the E2A proteins. Evidence implicating the tumor suppressor function of E2A has also come from the studies of the TAL family of oncogenes (reviewed by Baer, 1993). The TAL proteins, including Tal1, Tal2 and Lyl1, are all bHLH proteins that can dimerize with E2A proteins and bind to E boxes. Aberrant expression of the Tal1 gene, for example, has been found in 70% of human acute lymphoblastic leukemia (T-ALL) samples. Although Tal1 can form heterodimers with E47 to bind to DNA, the heterodimers potentiate the transcription of their target genes very poorly as compared to E47 homodimers. We have shown that the poor transactivation by

E47/Tal1 heterodimers is due to the incompatibility of the activation domains present in E47 and Tal1 (Park and Sun, 1998). In mammary epithelial cells, overexpression of Id-1 has been found to block the induced differentiation of a mouse mammary epithelial cell line, SCp2, which has been exposed to basement membrane and lactogenic hormones (Desprez et al., 1995). Moreover, these Id1 expressing SCp2 cells showed invasion of the basement membrane and resumption of cell proliferation. The same researchers then reported that Id-1 overexpression stimulates the expression of two novel polypeptides with gelatinase activities. It was therefore proposed that Id-1 expression may be related to the metastasis of breast cancer (Desprez et al., 1998). We had obtained preliminary evidence that Id-1 and Id-2 appear to be overexpressed in a fraction of breast cancer samples by using RT-PCR analyses. Based on these data, we have hypothesized that overexpression of Id proteins may lead to the inhibition of E2A function, which may then interfere with the normal process of mammary cell differentiation and tumor suppression. To test this hypothesis, we have proposed three objectives to determine if Id is a causal factor in breast cancer, to examine the regulation of Id gene expression, and to identify bHLH proteins functioning in breast cancer cells

## BODY

### A. To determine if Id is a causal factor in breast cancer

To test the effect of Id proteins on tumorigenesis and mammary gland development, we have generated transgenic mice carrying the Id-1 gene under the control of the mouse mammary tumor virus (MMTV) long terminal repeat (LTR). The Id1 cDNA was cloned into a modified MMTV transgenic vector obtained from Dr. B. Weinstein (Columbia University), which has been shown to direct high levels of expression in mammary tissues and low levels of expression in salivary gland and some male reproductive tissues (Yao and Weinstein, personal communication). We initially obtained six independent transgenic lines, of which two lines did not give any progenies, one line showed no expression, and three lines exhibited some levels of expression of the transgene upon preliminary analysis. Despite Id-1 expression detected in three lines of transgenic mice, no tumors were found in any of the transgenic mice after observation for up to 12 months. These transgenic mice have also been crossed with transgenic mice expressing the Her2/neu oncogene under the control of MMTV LTR. The resulting double transgenic mice did not develop any tumor. Therefore, we are tempted to conclude that Id-1 expression in our transgenic mice is not sufficient to cause breast cancer.

### B. Detection of Id-1 overexpression in human breast cancer samples.

As a project planned to be carried out in Year 2, we proposed to examine human breast cancer samples for Id-1 overexpression by using the in situ RNA hybridization technique. The major challenge in this experiment is that in situ hybridization has to be carried out using archived and formalin fixed tissue imbedded in paraffin. We initially used dioxigenin-labeled antisense RNA probes for hybridization followed by chromogenic detection with anti-dioxigenin antibodies directly conjugated with alkaline phosphotase, or in combination with secondary antibodies conjugated with alkaline phosphotase. After many attempts, we found that the non-specific background as judged by the signals generated by using the sense probes on these archived and formalin fixed tissues is too high for the results to be reliable. We thus switched to $^{35}$S-labeled RNA probes and the background was significantly reduced. We have used antisense β-actin probes as positive controls. A total of 26 breast carcinomas were analyzed for Id-1 overexpression. Eight samples are excluded due to poor RNA preservation demonstrated by very week signal of β-actin. Nine out of 18 breast carcinoma samples showed overexpression of Id-1. Representative samples with and without Id-1 overexpression are showed in Figure 1.

Table 1 summarizes the results of Id-1 expression along with clinical data. Id-1 overexpression is detected in 50% of human breast carcinoma samples, either ductal or lobular carcinoma. Id-1 overexpression is more frequently associated with younger patients (mean age = 49) compared to the mean age of patients with Id-1 negative carcinoma (mean age = 60). However, due to limited

number of samples, the observed difference in mean age is not statistically significant.

Previous studies on human breast cancer cell line have suggested that Id-1 expression may suppress mammary epithelial cell differentiation and promote invasion (Desprez et al., 1995, Desprez et al., 1998). We have therefore evaluated the relationship between Id-1 overexpression and pathological grade of breast carcinoma or the rate of lymph node metastasis. Our results showed that carcinomas with or without Id-1 overexpression have very similar pathological grade (7.44 vs. 7.43) and lymph nodes metastasis rate (83% vs. 86%).

In NIH 3T3 fibroblasts, overexpression of E47 or incubation with Id antisense oligonucleotides arrests cell cycle at the G1 to S phase transition (Peverali, et al., 1994; Hara et al., 1994; Hara et al., 1994). We thus attempted to determine whether Id-1 overexpression was associated breast carcinoma proliferation. We examined the relationship between Id-1 overexpression and mitotic activity of human breast carcinoma, and found no significant difference in mitotic figures between carcinomas with (mean = 9.2) and without (mean = 8) Id-1 expression.

Taken together, we have demonstrated that Id-1 is overexpressed in 50% of human breast carcinoma samples. However, Id-1 overexpression is not associated with generally applied clinical prognostic parameters such as pathological grade, lymph node metastasis, tumor size, and mitotic activity. It remains to be further investigated whether Id-1 overexpression influences the induction or progression of human breast carcinoma or whether it can be used as a molecular parameter for prognosis.

## C. Investigation of mechanisms involved in Notch1-mediated E2A degradation.

Since we have not established a causal effect of Id1 overexpression on breast cancer development using transgenic mouse models and in human breast cancer studies, we explored other mechanisms that may lead to down-regulation of E2A function, such as protein turnover. Electrophoretic mobility shift assays did not reveal any complexes that may contain heterodimers between E2A and other bHLH proteins as in the case of muscle or neuronal tissue. In fact, no significant amounts of complexes were detected using breast cancer cell lines (Fig. 4, 2000 report). Instead, as described above, E2A proteins were found rapidly degraded in breast cancer cell lines, MDA-231 and T47D, compared to a B cell line, WEHI-231 (Fig. 2A, 2000 report). E2A proteins were also more rapidly degraded in proliferating primary mammary epithelial cells compared to freshly isolated epithelia cells (Fig. 2B, 2000 report). Recently, we discovered that activated Notch1 accelerated the degradation of E2A proteins. Interestingly, the locus of Notch-4 was found to be a common integration site for the mouse mammary tumor virus in mouse mammary tumors, which results in the ectopic

expression of the intracellular domain of Notch-4 (reviewed by van Leeuwen and Nusse, 1995). Expression of this "activated" Notch-4 in mammary epithelium has profound effects on mammary gland development and tumorigenesis (Jhappan et al., 1992). Therefore, Notch-mediated E2A degradation might be of great significance to breast cancer development, which prompted us to further investigate the mechanisms underlying E2A degradation induced by Notch1.

As shown in Fig. 2, the transactivating activity of E47 is inhibited by co-transfection of a plasmid expressing the intracellular domain of human Notch1 (activated form), TAN1, in a dose dependent manner. However, this reduction in transactivating activity was attributed to decreased levels of E47 protein present in the co-transfected cells. We then mapped the region in E47 responding to Notch1-mediated degradation to a stretch of highly conserved sequences among several E2A like proteins (Fig. 3). Deletion of this sequence rendered E47 insensitive to Notch1-mediated degradation (Fig. 4). Furthermore, two MAP kinase recognition sites were found in this sequence. Mutations in these sites also resulted in stabilization of the protein (Fig. 5). Consistently, expression of a constitutively active MEK synergized with Notch1 in E47 degradation, while treatment with inhibitors of MAP kinase kinase (MEK) abolished Notch1 induced degradation of E47 (Fig. 6). Similar to that found in breast cancer cells (Fig. 5, 2000 report), the degradation of E47 could be inhibited by proteasome inhibitors (Fig. 7). Taken together, it appears that degradation of E47 may involve ubiquitin-mediated pathways that are often precisely regulated in response to various stimuli and in various cell types.

In summary, these results suggest that in mammary epithelial cells, E2A proteins, though expressed at a high level, are inactivated through mechanisms such as proteasome-mediated degradation. The biological significance of this novel regulatory mode of E2A function remains to be understood. It is also not clear whether E2A is constitutively inactivated or it functions to suppress excessive cell proliferation in a temporal manner when the need arises. If the latter is true, loss of E2A function under certain circumstances could potentially lead to breast cancer. It is also possible that E2A gene products, E12 and E47, may be some of the downstream effectors of the Notch signaling pathway, and degradation of these tumor suppressors contribute to breast cancer formation.

## KEY RESEARCH ACCOMPLISHMENTS

- Id-1 transgenic mice generated but no tumor formation was found in these mice.
- Id-1 overexpression detected in 50% of human breast cancer samples.
- E2A proteins found to be rapidly degraded through a proteasome-mediated pathway in primary mammary epithelial cells and breast cancer cell line, MDA-MB-231.
- Activated Notch is found to trigger the degradation of E2A proteins, which requires phosphorylation at the MAP kinase sites in an evolutionarily conservation domain in E2A proteins.
-

## REPORTABLE OUTCOMES

--- Manuscripts, abstracts presentations:
Abstract for a poster at DoD Breast Cancer Program "Era of Hope" meeting, Atlanta, GA 2000.
Manuscript in preparation "Notch1-mediated degradation of E2A proteins requires phosphorylation at the MAP kinase sites in a conserved domain shared by all E proteins".

--- patents and license applied for and/or issued: None.

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--- development of cell lines, tissue or serum repositories:
*Id-1 transgenic mouse lines, pYY-Id1-30 and pYY-Id1-59.*

--- informatics such as databases and animal models, etc.

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## (9) CONCLUSIONS

This study has led to the discovery Id-1 overexpression in a large fraction of human breast cancer. In addition, a novel mechanism of E2A protein degradation has also been found in mammary epithelial cells. Activation of the Notch signal pathway, known to cause breast cancer, has been shown to accelerate the degradation of E2A proteins through a similar mechanism. These findings thus suggest means to inactivate the E2A transcription factor and tumor suppressor, which could affect breast cancer development.

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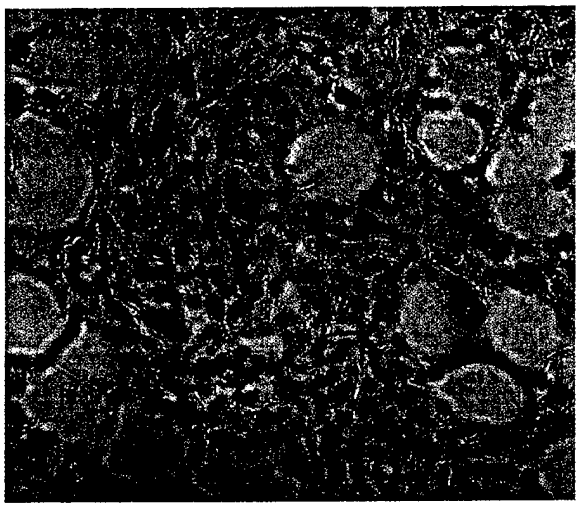
**APPENDICES:**

(a) Figures 1-7.

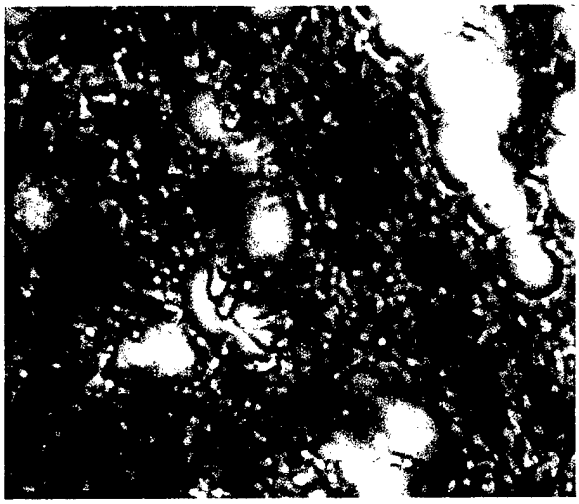
(b) Table 1

(c) CV of the PI.

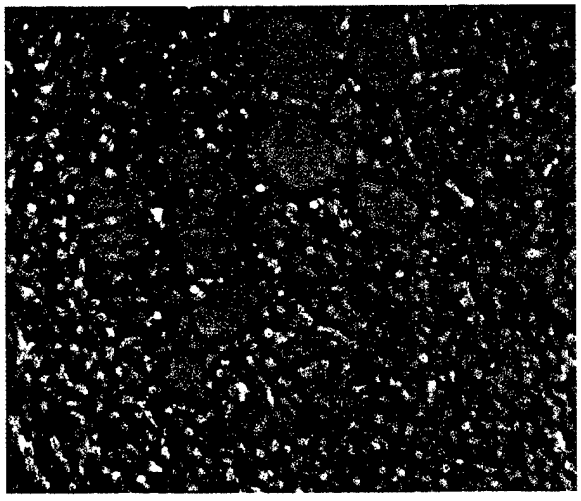
Figure 1



A. H&E section human breast carcinoma. (10X)



B. In situ hybridization with Id-1 anti sense probe. (10x)



C. In situ hybridization with Id-1 sense probe. (10x)

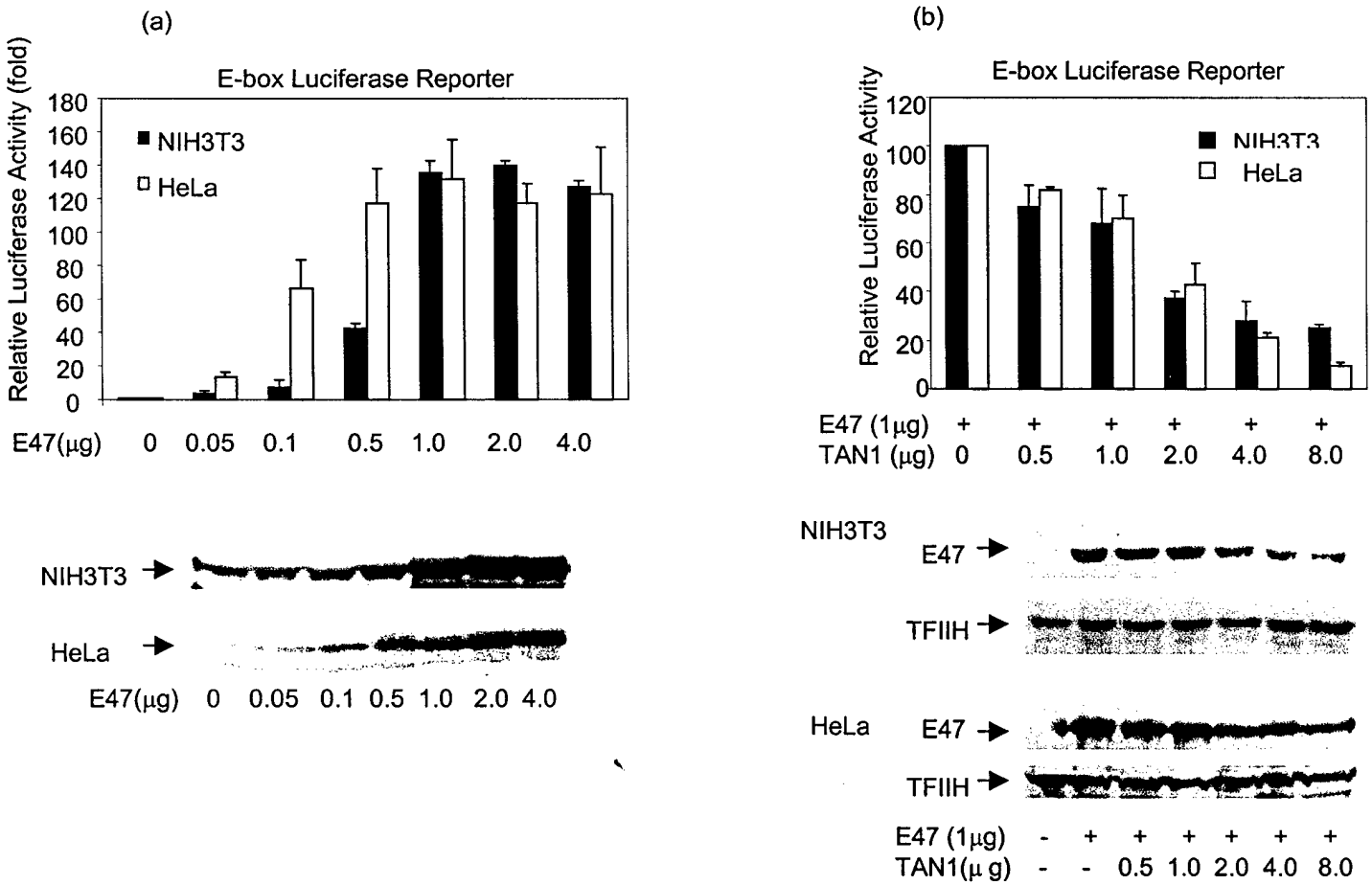



Figure 2. Notch-mediated inhibition of the transactivation by E47 and degradation of E47. (a) Dose-dependent transactivation by E47 in transiently transfected NIH3T3 and HeLa cells. Different amounts of E47 expressing plasmid DNA were co-transfected with E-box luciferase reporter and CMV-LacZ plasmids. Luciferase and β-galactosidase activities were measured 36 hours after transfection. Luciferase activities were normalized against β-galactosidase activities. Whole cell lysates were also analyzed using western blots with antibodies against E47. (b) E47 transactivating activities and protein levels in the presence of Notch1 intracellular domain (TAN1) in cotransfection experiments as described in (a).

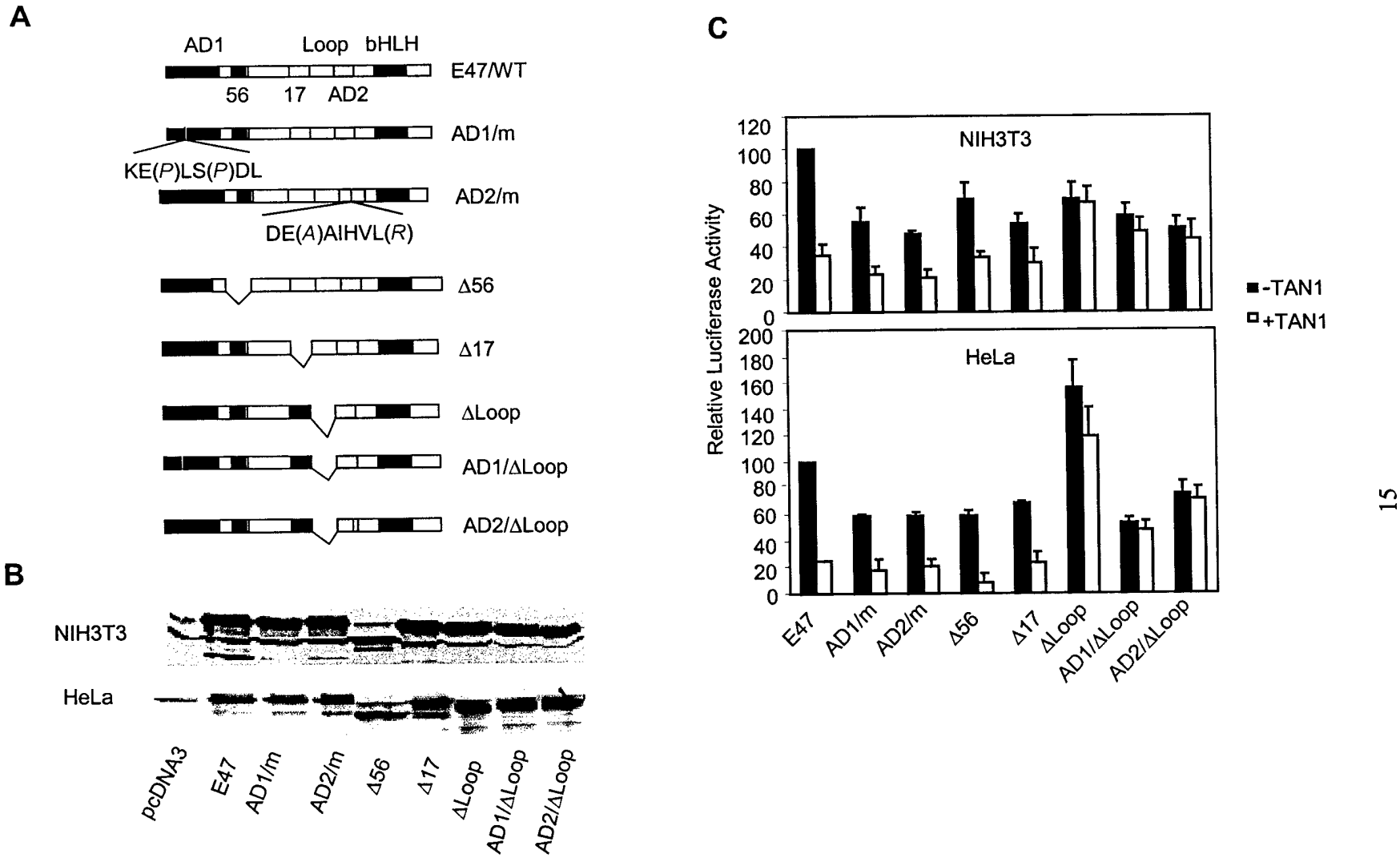



Figure 3. Identification of Functional Domain Responsible for Notch1-Mediated Transcriptional Inhibition On E47 Protein. Mutants of E47 were generated and diagramed in (a) and their expression was confirmed using western blots shown in (b). The transactivating activities were determined in the absence and present of TAN1 as described in the legend of Fig. 2a.

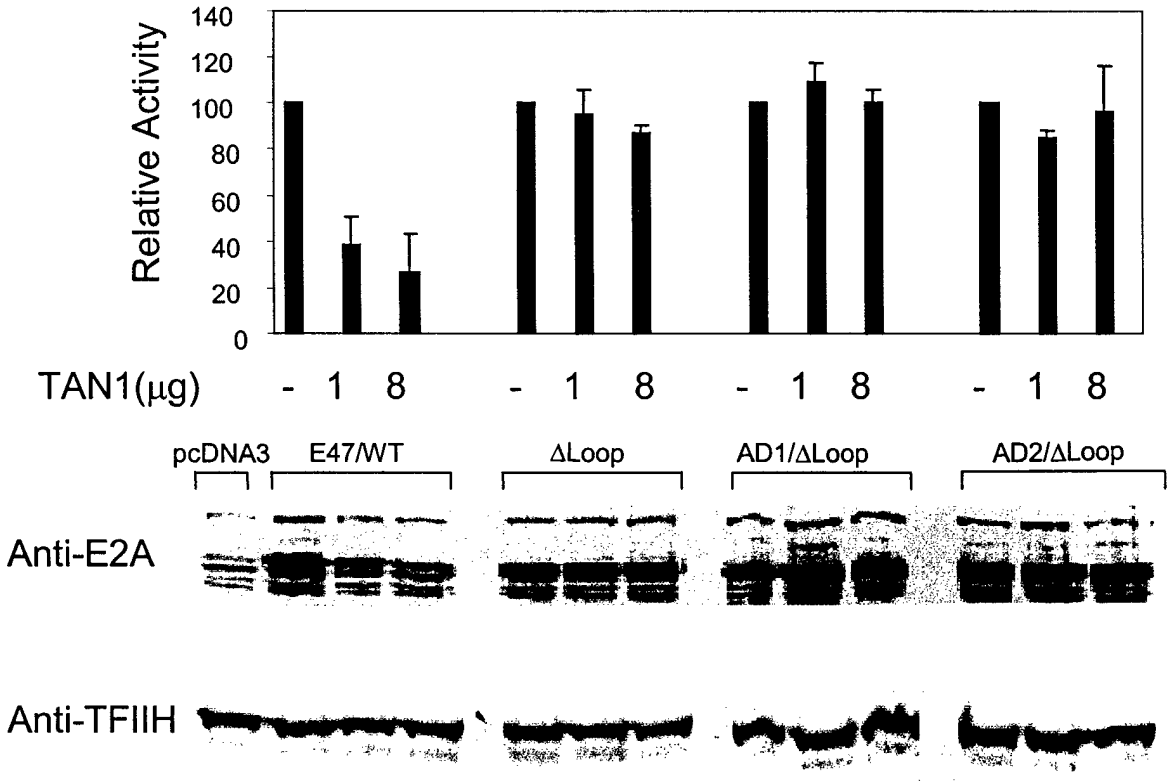



**Figure 4. Loop Deletion Renders the Protein Resistant to the Notch1-Mediated Degradation**. HeLa cells were transfected in duplicates with 1 μg of wild type or mutant E47 and the indicated amounts of TAN1 together with an E-box luciferase reporter gene and a CMV-LacZ construct. One set of the transfectants was used to measure luciferase and β-galactosidase activities and the other set used for western blot analyses. Relative activities represent luciferase activities normalized against β-galactosidase activities. The amount of TFIIH is used as a control for the amount of Protein loaded in each lane.

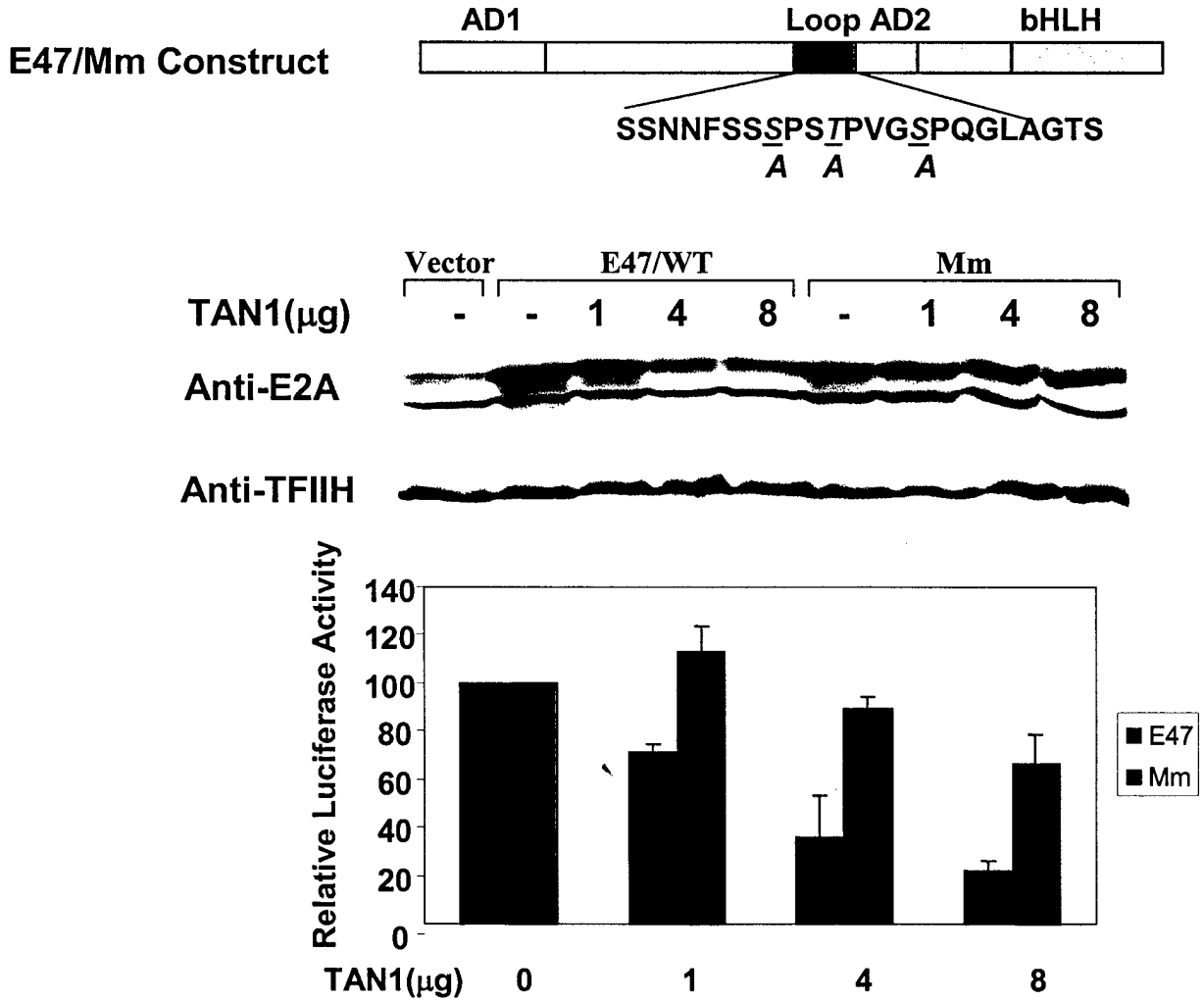



Figure 5. Mutation of the MAPK Sites in the Loop Region Renders the Protein Resistant to the Notch1-Mediated Degradation. Wild type and Mm mutant E47 were used in transfection experiments as described in the legend of Fig. 4.

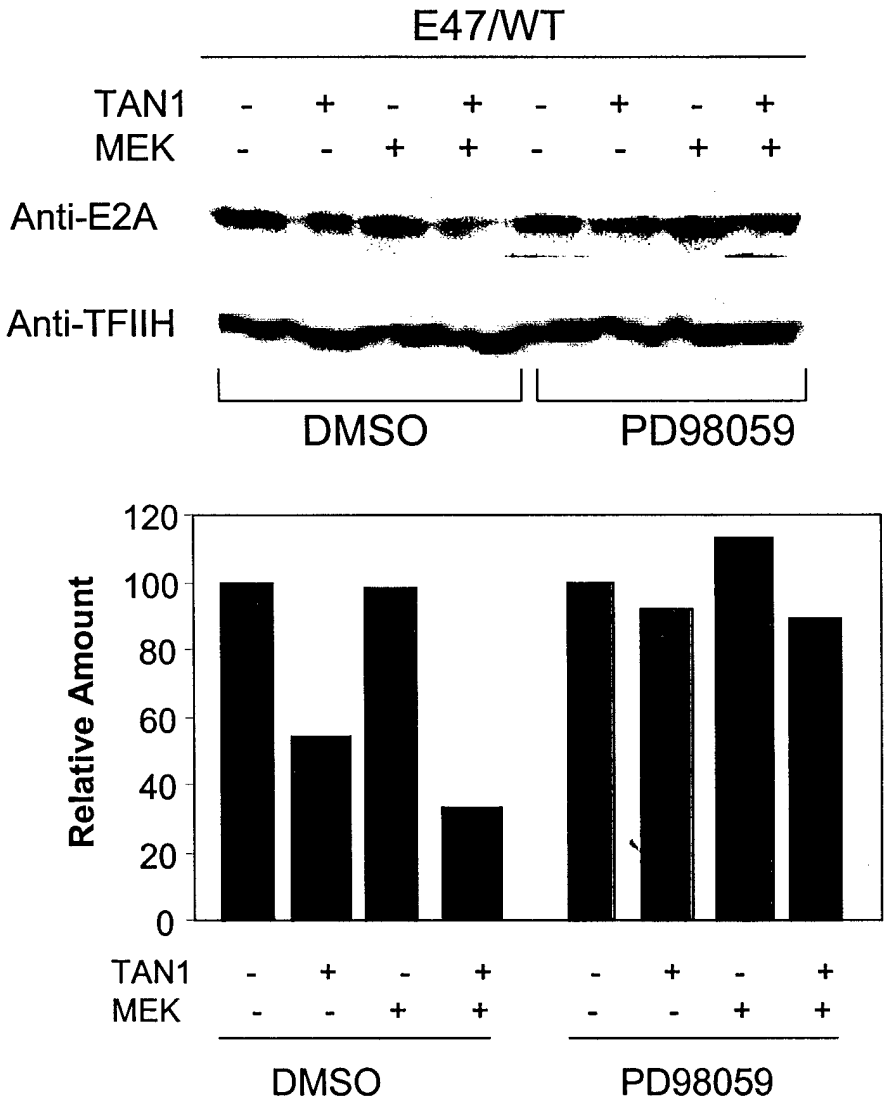



Figure 6. Western blot analyses of transfected cells with 3 μg of plasmids expressingTAN1, MEK or both along with E47. MEK is a constitutively active mutant of MEK1. Transfected cells were treated with EMK inhibitor PD98059 or its vehicle, DMSO for 4 hours at the end of 36-hr transfection. The amount of E47 proteins detected with an ECL reagent was quantified on a luminImager and normalized with the amount of TFIIH on the same membrane. Relative amount represent the percentage of E47 protein compared to cells transfected E47 expressing plasmid alone.

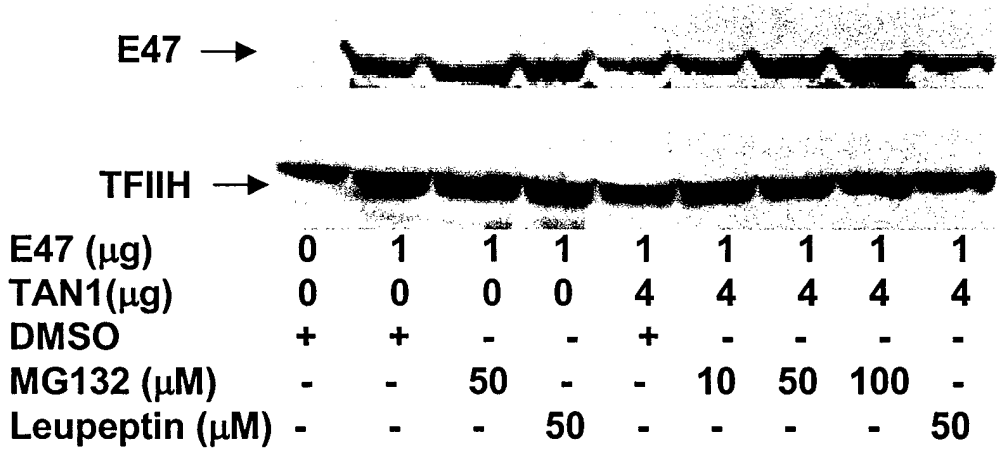





Figure 7. Proteasome Inhibitor Blocks Notch1-Induced Degradation of E47 Protein. Western blot analysis of cells transfected with indicated constructs and treated with indicated agents for 4 hours at the end of the transfection experiment.

Table 1. Id-1 expression and clinical data of human breast carcinomas

| Sample | Id1 | Age | tumor type | Grade | Size | LN | Mitosis |
|---|---|---|---|---|---|---|---|
| 98-59 | - | 87 | d | 8 | 2.3 | | 4 |
| 98-4575 | - | 62 | l | | 2.4 | y | 2 |
| 98-11562 | - | 53 | d | 5 | 1.3 | n | 5 |
| 98-11933 | - | 41 | l | | 6.5 | y | 12 |
| 98-12755 | - | 56 | d | 7 | 3 | y | 3 |
| 98-12037 | - | 46 | d | 8 | 2.2 | y | 15 |
| 98-17298 | - | 48 | d | 7 | 10 | y | 3 |
| 99-517 | - | 75 | d | 9 | 1.6 | y | 20 |
| 99-726 | - | 69 | d | 8 | 1.5 | y | 8 |
| **Average** | | 59.7 | | 7.4 | 2.7 | | 9 |
| | | | | | | | |
| 98-3800 | + | 49 | d | 8 | 1.6 | | 6 |
| 98-9336 | + | 47 | d | 5 | 6 | y | 2 |
| 98-14396 | + | 48 | d | 7 | 10 | y | 3 |
| 98-14936 | + | 38 | d/l | 9 | 2 | y | 34 |
| 98-20976 | + | 53 | d | 6.5 | 3.5 | | 4 |
| 99-760 | + | 56 | d | 8 | 2.5 | y | 9 |
| 99-1336 | + | 33 | l | 6.5 | 2.2 | | 3 |
| 99-1769 | + | 76 | d | 8 | 1.2 | y | 17 |
| 99-4279 | + | 52 | d | 7 | 2.1 | n | 2 |
| **Average** | | 49 | | 7.4 | 3.4 | | 8 |

D = ductal; l = locular; LN = lymph node metastasis; size is in cm.

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| 1998 - 1999 | Associate Professor<br>Department of Cell Biology,<br>New York University School of Medicine. |
| 1999 – present | Associate Member<br>Immunobiology & Cancer Research Program<br>Oklahoma Medical Research Foundation. |
| 1999 – present | Adjunct Associate Professor<br>Department of Microbiology and Immunology Program<br>Department of Cell Biology<br>University of Oklahoma Health Science Center |

***PROFESSIONAL SOCIETIES:***

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| 1991 | Member of the American Association for the Advancement of Science. |
| 1992 | Member of the American Society for Microbiology. |
| 1999 | Member of the American Association of Immunologists. |
| 1994 | Member of the Society of Chinese Bioscientists in America.<br>Served in the Council (1996) and the Membership Committee (1994-1996), Society of Chinese Bioscientists in America. Secretary-elect (2002-2003). President of the Tri-State Chapter (1997-1998), |
| 1998 | Member of Ray Wu Society for Life Sciences.<br>Board of the Directors (1998-2002). |

***AWARDS:***

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| 1981 | CUSBEA (China-United States Biochemistry Examination and Application) exchange student. |
| 1989 - 1991 | Cancer Research Institute Postdoctoral Fellowship. |
| 1991 - 1996 | Markey Scholar (Supported by a grant from The Lucille P. Markey Charitable Trust Foundation to NYUSM). |
| 1992 - 1993 | Whitehead Presidential Fellowship. |
| 1992 - 1996 | Cancer Research Institute Investigator Award. |
| 1994 - 1998 | Irma T. Hirschl Trust Career Scientist Award. |

***GRANTS:***

**1 R01CA77553 (Xiao-Hong Sun)** 1/1/99 – 12/31/03
NCI/NIH
The molecular mechanism of T cell leukemogenesis induced by TAL.

**1 R01AI33597-08 (Xiao-Hong Sun)** 5/1/01 – 4/30/06
NIAID/NIH
Regulation of the Id genes in lymphocyte development.

**IDEA grant (Xiao-Hong Sun)** 5/1/98 to 10/31/01
US Army
The role of Id proteins in breast cancer.

**1 P20 RR15577 (J. Donald Capra)** 9/30/00 to 8/31/05
NIH
The role of bHLH proteins in human lymphopoiesis.

***OTHER PROFESSIONAL ACTIVITIES:***

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| 1991 - present | Reviewer for "Immunity", "Journal of Virology", "Molecular and Cellular Biology", "Nucleic Acids Research", "Journal of Cellular Physiology", and Journal of Neuroscience" . |
| 1993 | Reviewer for grants received by the United States Army Medical Research and Development Command for research on breast cancer. |
| 1995 -1997 | Reviewer and site-visitor for two NIH (NIDDK) program project grants. |
| 2000 | Ad Hoc reviewer for the Immunobiology Study Section. |
| 1995 | Invited speaker at the Sixth International Symposium of the Society of Chinese Bioscientists in America, Vancouver, Canada. |
| 1996 | Speaker at the Joint Symposium of American Association of Immunologists, American Association of Biochemists and American Associate of Investigative Pathologists, New Orleans, LA. |

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| 1997 | Invited speaker at the Seventh International Symposium of the Society of Chinese Bioscientists in America, Toronto, Canada |
| 1998 | Invited speaker at Gene Therapy/Molecular Biology International Conference, Heraklion island of Crete, Greece. |
| 1998 | Invited speaker at New England Immunology Conference, Woods Hole, MA. |
| 1999 | Invited speaker at Gene Therapy/Molecular Biology International Conference, Redwood City, CA. |
| 1999 | Session chair and speaker at the Eighth International Symposium of the Society of Chinese Bioscientists in America, Hong Kong. |
| 2001 | Speaker at the Experimental Biology 2001 Conference, Orlando, FL. |
| 1994 - present | Seminar speaker at Thomas Jefferson Cancer Center, Harvard Medical School, Yale Medical School, NIH, UCLA and UMDNJ. |

***TEACHING EXPERIENCE:***

**Graduate and Medical School Courses at New York University:**

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| 1992 | Nucleic Acids Core Course for graduate students. |
| 1992 | Molecular and Cellular Biology |
| 1994 - 1998 | Cell Biology of tissues and organs (Histology). |

**Graduate and Medical School Courses at OUHSC:**

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| 2000 | Advanced Immunology (one class and one exam). |
| 2000 | Immunology discussion groups for medical students. |

**Graduate School Training:**

1991 - 2000 New York University School of Medicine.

Thesis advisor: Four graduate students and one MD-PhD student. (all received Ph.D.).

| Student | Current position |
|---|---|
| Sittichoke Saisanit, | Scientist, Hoffman La Roch, NJ |
| Sumangala Prabhu | Associate, CSC Healthcare, Inc. |
| Steven T. Park, | Resident, University of California at Irvine |
| Antoaneta Vladimirova | Continuing education in bioinformatics |
| Min Xu | Postdoctoral fellow, OMRF |

Thesis committee member: Five graduate and one MD-PhD students.

2000-present University of Oklahoma Health Sciences Center

Ph.D. Student:
Seung-Hwen Kim

Committee member:
Joseph Jeong Department of Microbiology and Immunology
Jiaxue Huang Department of Microbiology and Immunology

***BIBLIOGRAPHY:***


1. Tso, J. Y., **Sun, X.-H.,** Kao, T.-h., Reece, K., and Wu, R. (1985). Isolation and characterization of rat and human glyceraldehyde 3-phosphate dehydrogenase cDNAs: genomic complexity and molecular evolution of the gene. *Nucl. Acids Res.* 13: 2485-2502.

2. Tso, J. Y., **Sun, X.-H.** and Wu, R. (1985) Structure of two unlinked *Drosophila melanogaster* glyceraldehyde 3-phosphate dehydrogenase genes. *J. Biol. Chem.* 260: 8220-8228.

3. **Sun, X.-H.,** Lis, J. and Wu, R. (1988) The positive and negative transcriptional regulation of the *Drosophila Gapdh*-2 gene. *Genes Dev.* 2: 743-753.

4. **Sun, X.-H**., Tso, J. Y., Lis, J. and Wu, R. (1988) Differential regulation of the two glyceraldehyde-3-phosphate dehydrogenase genes during *Drosophila* development. *Mol. Cell. Biol.* 8: 5200-5205.

5. **Sun, X.-H**., and Baltimore, D. (1989) Human immunodeficiency virus tat-activated expression of poliovirus protein 2A inhibits mRNA translation. *Proc. Natl. Acad. Sci. USA* 86: 2143-2146.

6. Kamps, M. P., Murre, C., **Sun, X.-H**., and Baltimore D. (1990) A new homeobox gene contributes the DNA-binding domain of the t(1:19) translocation protein in pre-B ALL. *Cell* 60: 547-555.

7. **Sun, X.-H**. and Baltimore, D. (1991) An inhibitory domain of E12 transcription factor prevents DNA binding in E12 homodimers but not in E12 heterodimers. *Cell* 64: 459-470.

8. **Sun, X.-H**., Copeland, G., Jenkins, N. and Baltimore, D. (1991) The Id proteins Id1 and Id2, selectively inhibit DNA binding by one class of helix-loop-helix proteins. *Mol. Cell. Biol.* 11: 5603-5611.

9. **Sun, X.-H.** (1994) Constitutive expression of the Id1 gene impairs mouse B cell development. *Cell* 79: 893-900.

10. Saisanit, S. and **Sun, X.-H.** (1995) A novel enhancer, the pro-B enhancer, regulates Id1 gene expression in progenitor B-cells. *Mol. Cell. Biol.* 15: 1513-1521.

11. Laure, T. M., Starovasnik, M. A., Weintraub, H., **Sun, X.-H.** and Klevit, R. E. (1995) MyoD forms micelles which can dissociate to form heterodimers with E47. Implications of micellization on function. *Proc. Natl. Acad. Sci. USA.* 92: 11824-11828.

12. Mahajan, M. A., Park, S. T., and **Sun, X.-H.** (1996) Association of a novel GTP-binding protein, DRG, with TAL oncogenic proteins. *Oncogene* 12: 2343-2350.

13. Vitola, S. J., Wang, A. and **Sun, X.-H.** (1996) Substitution of basic amino acids in the basic region stabilizes DNA binding by E12 homodimers. *Nucleic Acids Res.* 24:1921-1927.

14. Saisanit, S. and **Sun, X.-H.** (1997). Regulation of the pro-B-cell-specific enhancer of the Id1 gene involves the C/EBP family of proteins. *Mol. Cell Biol.* 17: 844-850.

15. Chen, B., Han, B. H., **Sun, X.-H.** and Lim, R. W. (1997). Inhibition of muscle-specific gene expression Id3: requirement of the C-terminal region of the protein for stable expression and function. *Nucl. Acid. Res.* 25: 423-430.

16. Prabhu, S., Ignatova, A., Park, S. T. and **Sun, X.-H.** (1997). Regulation of the expression of cyclin-dependent kinase inhibitor p21 by E2A and Id proteins. *Mol. Cell. Biol.* 17: 5888-5896.

17. Dang, W., **Sun, X.-H.** and Sen, R. (1998). ETS mediated cooperation between basic helix-loop-helix motifs of the immunoglobulin mu heavy chain gene enhancer. *Mol. Cell. Biol.* 18: 1477-1488.

18. Park, S. T. and **Sun, X.-H.** (1998). The Tal1 Oncoprotein inhibits E47-mediated transcription: Mechanism of Inhibition. *J. Biol. Chem.* 273: 7030-7037.

19. Park, S. T., Nolan, G. P. and **Sun, X.-H.** (1999). Growth inhibition and apoptosis due to restoration of E2A activity in T cell acute lymphoblastic leukemia cells. *J. Exp. Med.*,189: 501-508.

20. Pan, L., Sato, S., Frederick, J. P., **Sun, X.-H.** and Zhuang, Y. (1999) Impaired immune responses and B cell proliferation in mice lacking the Id3 gene. *Mol. Cell. Biol.* 19: 5969-5980.

21. Kim, D., Peng, X., and **Sun, X.-H.** (1999). Massive apoptosis of thymocytes in T cell deficient Id1 transgenic mice. *Mol. Cell. Biol.* 19: 8240-8253.

22. Yao Y., Doki, Y., Jiang, W., Imoto, M., Venkatraj, V. S., Warburton, D., Santella, R. M., Lu, B., Yan, L., **Sun, X.-H.,** Su, T., Luo, J., and Weinstein, I. B. (2000) Cloning and characterization of DIP1, a novel protein that is related to the Id family of proteins. Exp Cell Res 257:22-32.

23. Kim, D. Xu, M., Nie, L, Peng, X.-C., Jimi, E., Voll, R. E., Nguyen, T, Ghosh, S. and **Sun X.-H.** Helix-Loop-Helix Proteins Regulate Pre-TCR and TCR Signaling through Modulation of Rel/NF-κB Activities. Immunity, in press.

24. Xu, M., Nie, L. and **Sun, X.-H.** Recruitment of histone deacetylase 1 in STAT5-mediated transcription of the Id-1 gene. Submitted.

25. Nie, L., Xu, M., Vladimirova, A. and **Sun, X.-H.** Notch1-mediated degradation of E2A proteins requires phosphorylation at the MAP kinase sites in a conserved domain shared by all E proteins. Manuscript in preparation.