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14. ABSTRACT
Estrogen-receptor alpha (ERα)-positive Progesterone receptor negative (ER+/PR-) breast ductal carcinomas comprise approximate15-25% of human breast cancers. However, molecular mechanisms underlying the development of this subtype of breast cancer remain poorly understood. Using genetically-engineered Tip30 knockout mice generated in our laboratory, we previously demonstrated that Tip30 deletion results in development of tumors in several tissues and ductal hyperplasia in the mammary glands. This project is to study the molecular mechanism(s) underlying ER+/PR- breast tumorigenesis. Specifically, we proposed to determine genetic and epigenetic alterations in the initiation and progression of ER+/PR- mammary tumors arising in Tip30-/-/MMTV-neu mice. Here we show that Tip30 deletion in MMTV-Neu mice significantly accelerates the formation of ER+/PR- mammary tumors. An unbiased DNA microarray analysis revealed that Tip30 deletion resulted in increased activation of cAMP-mediated signaling, EGF signaling, IGF signaling and PI3K/AKT signaling in ER+/PR- mammary tumors. Taken together, our data suggest that inactivation of TIP30 may contribute to the development of ER-positive and PR-negative breast cancers through activation of EGF and IGF signaling pathways.

15. SUBJECT TERMS
Estrogen receptor, Progesterone receptor, breast cancer, tumorigenesis

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Table of Contents

Introduction:

To date, the mechanisms underlying de novo and acquired ER+/PR- breast cancer remain poorly defined(1). Thus, elucidation of the molecular basis of ER+/PR- breast tumor development has the potential to reveal new therapeutic targets in the treatment, or even prevention, of the resistance to anti-estrogen therapy in patients with breast cancer(2, 3). TIP30 is a human cellular 30kDa protein that was purified as a HIV-1 Tat interacting protein (4)and is expressed in various tissues in humans and mice(4, 5). Using genetically-engineered mouse models generated in our laboratory, we have demonstrated that *Tip30* deletion results in development of ductal hyperplasia and tumors in mouse several tissues (6, 7). Recently, we made novel observations that *Tip30* deletion accelerates mammary tumorigenesis induced by MMTV-Neu oncogene and mammary tumors consisting of ER-positive and PR-negative (ER+/PR-) luminal epithelial cells. This project is to study the molecular mechanism(s) underlying ER+/PR- breast tumorigenesis. Specifically, we will determine genetic and epigenetic alterations in the initiation and progression of ER+/PR- mammary tumors arising in Tip30-/-/MMTV-neu mice; and we will also evaluate IGF-I and Wisp-2 as potential therapeutic targets for ER+/PR- mammary tumors developed in *Tip30-/-* MMTV-neu mice. Results generated during the first year indicate that Tip30 loss accelerates ER+/PR- mammary tumors in MMTV-Neu mice through EGF and IGF-1 mediated pathways.

Body:

Task 1. **Determine specific genetic and epigenetic alterations in the initiation and progression of ER+/PR- mammary tumors arising in Tip30-/-/MMTV-neu and Tip30+/-/MMTV-neu mice.** We have completed the proposed experiments in Task 1; a and b, and most of experiments in Task1 d, which were proposed to complete in the first year. We have demonstrated that Tip30 loss accelerated mammary tumorigenesis in MMTV-Neu mice (unpublished data). In order to further characterize mammary tumors developed form these mice, we have generated a cohort of MMTV-neu/ *Tip30+/+*, MMTV-neu/ *Tip30+/-* and MMTV-neu/ *Tip30*$^{-/-}$ mice. We have been monitoring these mice for the development of mammary tumors and collected mammary tumors that developed in these mice for pathological analysis and further investigations. In order to determine expression pattern of ERα, PR-A and PR-B proteins in these mammary tumors and their adjacent mammary tissues, the tumors were subjected to immunofluorescence staining with antibodies specific for ER, PR-A and PR-B. The tumor cells in all seven *Neu+/Tip30*$^{-/-}$ tumors examined were ERα positive and PR (PR-A and PR-B) negative (ER+/PR-) (table 1). The tumor cells from six of seven *Neu+/Tip30*$^{+/+}$ tumors examined were both ERα and PR negative (ER-/PR-) (Table 1). In addition, we observed that the adjacent mammary glands contained ER-positive and PR-A-positive ductal cells in both *Neu+/Tip30*$^{-/-}$ and *Neu+/Tip30*$^{+/+}$ tumors while no PR-B-positive ductal epithelial cells were detected. The antibodies for PR used in immunofluorescence analysis are able to detect both PR-A and PR-B. This data suggests that *Neu+/Tip30*$^{-/-}$ female mice spontaneously develop ER+/PR- mammary tumors and *Tip30*$^{-/-}$ spontaneously develop ER+/PR+ or ER+/PR- mammary tumors. Moreover, some of these tumor

tissues were used for making RNA probes for microarray analysis and the establishment of tumor cell lines in Task1d and Task2.

Table 1. ER and PR (A,B) expressions in murine mammary tumor

Animal No.	N	Tip30	ER α	PgRA	PgRB
648	+	-/-	+	-	-
942	+	-/-	+	-	-
924	+	-/-	+	-	-
923	+	-/-	+	-	-
1281	+	-/-	+	-	-
1288	+	-/-	+	-	-
1278	+	-/-	+	-	-
634	+	+/-	-	-	-
736	+	+/-	+	-	-
743	+	+/-	-	-	-
747	+	+/-	+	-	-
906	+	+/-	+	-	-
933	+	+/-	-	-	-
951	+	+/-	+	-	-
961	+	+/-	-	-	-
1131	+	+/-	-	-	-
1764	+	+/-	-	-	-
733	+	+/+	-	-	-
762	+	+/+	+	-	-
1047	+	+/+	-	-	-
1760	+	+/+	-	-	-
2-8-08-1	+	+/+	-	-	-
2-8-08-2	+	+/+	-	-	-
2-8-08-3	+	+/+	-	-	-
2-8-08-4	+	+/+	-	-	-

To identify genetic alterations in regulatory pathways and gene expression that would explain the observed phenotypes, we performed an unbiased microarray analysis to identify the genes differentially expressed between *Neu+/Tip30*$^{-/-}$ and *Neu+/Tip30*$^{+/+}$ tumors using the GeneChip® Mouse Gene 1.0 ST Array (Affymetrix) that contains 28,863 mouse genes and offers whole-transcript coverage. We found that 538 genes were changed more than 2-fold, which includes 181 genes were upregulated and 357 genes were downregulated. These genes are involved in ion and protein transportation, cell adhesion, cell proliferation and apoptosis signaling pathway. Ingenuity pathway analysis of altered gene profiles revealed that the top cancer-associated pathways affected by Tip30 deletion in Neu+ mammary tumors are cAMP-mediated signaling, EGF signaling, IGF signaling and PI3K/AKT signaling and G-protein coupled receptor signaling. These results are consistent with our previous findings that Tip30 loss increases expression of two growth factors, IGF-1 and Wsip2, in mammary epithelial cells. In addition, these results also implicate that Tip30 loss may accelerates an increased activation of Akt that is a common downstream target in these grow factor mediated signaling pathways.

Task 2. Evaluate IGF-I and Wisp-2 as potential therapeutic targets for ER+/PR- mammary tumors developed in *Tip30-/-* MMTV-Neu mice. We have completed Task2 a and b and potions of Task2 c. Given our previous observation that expression of IGF-1 and Wisp-2 was elevated in *Tip30*$^{-/-}$ mammary epithelial cells (8), we examined IGF-1 and Wisp-2 expression in the tumors from *Neu+/Tip30*$^{-/-}$, *Neu+/Tip30*$^{+/-}$, and *Neu+/Tip30*$^{+/+}$ mice with IHC analysis. Figure 1 shows in a representative comparison that the level of IGF-1 protein in *Neu+/Tip30*$^{-/-}$ tumors (scored as ++) appears to be higher than that in *Neu+/Tip30*$^{+/+}$ tumors (scored as +); IHC staining of IGF-1 and Wisp2 in the tumors are summarized in Table 2. We also used qRT-PCR to measure the mRNA levels of IGF-1 and Wisp2 in four mammary tumors (data not shown). These results indicate that IGF-1 and Wisp2 expression are increased in *Neu+/Tip30*$^{-/-}$ tumors

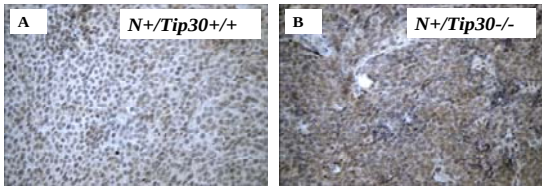

Fig.1. IGF-1 expression in mammary tumors. Paraffin sections of tumors from mice with the indicated genotypes were stained with anti-IGF-1. Brown stain indicates IGF-1 protein. Panel A: +; Panel B: ++.

Table 2. Immunohistochemical analysis of IGF-1 and Wisp2

Animal No.	Tip30	ERα	PR(A+B)	IGF-1	WISP-2
648	-/-	+	-	++	+
942	-/-	+	-	++	++
924	-/-	+	-	++	++
923	-/-	+	-	++	++
634	+/-	-	-	+	+
736	+/-	+	-	++	++
743	+/-	-	-	-	-
747	+/-	+	-	+	-
906	+/-	+	-	+	+
933	+/-	-	-	+	-
951	+/-	+	-	+	++
961	+/-	-	-	+	+
1131	+/-	-	-	+	+
1764	+/-	-	-	+	+
733	+/+	-	-	+	-
762	+/+	+	-	+	+
1047	+/+	-	-	-	+
1760	+/+	-	-	+	+

Key research Accomplishments:

1. Our data demonstrates th at Tip30 loss accelerates ER +/PR- mammary tumors in MMTV-Neu mice.
2. Our data su ggests that ER+/PR- mammary tumors arising in *Tip30*-null/MMTV-neu mice exhibit increased activation of EGF and IGF-1 pathways.

Reportable outcomes:

1. Part of this work was presented as a short talk at "Midwest Breas t Cancer Research Symposium" held at the University of Iowa from July 17 – 19, 2009.

2. Chengliang Zhang, Isam u Hoshino, Mikito Mori, Jill Pecha and Hua Xiao., The mechanism and role of TIP30 in m ammary tum origenesis. Midwest Breast Cancer Research Symposium. 2009. Abstract 32; pg 43.

3. A NIH RO1 grant application entitled "the role of a tumor suppressor in mammary tumorigenesis" is submitted partly based on work supported by this award

Conclusions: Our data suggest that Tip30 loss accel erates ER+/PR- mammary tumors in MMTV-Neu mice through EGF and IGF-1 mediated pathways.

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