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14. ABSTRACT

We have observed a dramatic increase of adipocyte-derived matrix protein collagen VI (COL6) level during cancer progression, particularly it relates to a discrete C-terminal domain of the alpha3 subunit of COL6. I have established 2 lines of transgenic mice which overproduce C-terminal domain, called "C5", of the COL6A3 (COL6A3-C5) under the control of MMTV promoter and crossed with MMTV-PyMT mice to see the C5 effects on mammary tumor progression in vivo. Our results indicate that COL6A3-C5 augments primary tumor growth and pulmonary metastasis in MMTV-PyMT mammary tumor mice model in vivo. Based on the cDNA microarray data with tumor tissues, COL6A3-C5 seems to be a signaling molecule regulating a kinase (or a phosphatase) activity which may be through a specific receptors that remains to be identified. Furthermore, treatment with COL6A3-C5 neutralizing antibodies in MMTV-PyMT mice protects mammary tumor progression. I have determined the effects of COL6 on the TZD mediated enhancement of cisplatin susceptibility with PyMT/COL6-/- mice. Tumor growth in PyMT/COL6-/- mice was significantly attenuated either by cisplatin or by a combination of cisplatin and TZD compared to PyMT/COL6+/+. To better understand the COL6 effects on drug resistance, I have tested COL6A3-C5 neutralizing antibodies in combination with cisplatin and TZD using the primary cultured tumor cells implantation system. To visualize the tumor progression in vivo, I generated MMTV-F635 transgenic mice which overproduce an infrared fluorescence protein specifically in the mammary epithelial cells. This allows me for the first time to monitor efficacy of treatment modalities longitudinally in mice.

15. SUBJECT TERMS

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INTRODUCTION

Subject:

The interactions between malignant ductal epithelial cells and the surrounding stromal cells play a crucial role in mammary tumor progression (*1*). The adipocyte is one of the predominant stromal cell types in the tumor microenvironment. The adipocyte is a highly active endocrine cell secreting numerous signaling molecules and profoundly shaping stromal-epithelial interactions (*2-3*). However, it remains unclear which specific adipocyte-derived factors are involved and how malignant cells are regulated by these factors *in vivo*. We have previously identified a prominent adipocyte-derived extracellular matrix (ECM) protein, type 6 Collagen (COL6), as an important stimulator of mammary tumor growth. It is highly up regulated in human breast cancer patients (*4-5*). Our previous work of COL6 knock-out (KO) mice in the background of MMTV-PyMT (mammary tumor virus-polyoma middle T antigen) demonstrated significantly attenuated rates of early hyperplasia and primary tumor formation (*5*).

Purpose:

In this project, I have focused on the carboxy-terminal domain of COL6 alpha3 subunit named COL6A3-C5, because I previously determined that the carboxy-terminal domain of COL6 is highly enriched in malignant tumor tissues relative to full length COL6. The COL6A3-C5 domain is cleaved off from the COL6 microfilament ECM structure upon secretion from the adipocyte (*6*). We believe that the adipocyte-derived, cleaved form of the C5 protein acts as a signaling molecule, influencing tumor growth and metastasis through various downstream signaling pathways. Therefore, I have explored the roles of **adipocyte-derived COL6A3-C5 as a novel mitogen** in mammary tumor progression *in vivo*. Furthermore, it has been suggested that increased levels of COL6 are involved in chemo-resistance to platinum-based therapeutic approaches in cancers, which is a widely used chemotherapeutic agent (*7-8*). The molecular mechanisms that explain platinum resistance in tumor cells remain largely unknown. Anti-diabetic agents, thiazolidinediones ("TZD's", PPARγ agonists) have been studied in human cancer therapy based on anti-mitogenic and terminal differentiation therapy as well as combination therapy with platinum-based regimen (*9-10*). Our observation that COL6 level is decreased by TZD treatment let us to investigate whether **COL6 is involved in the synergistic effects of TZD in combination with platinum-based therapy *in vivo*.**

Scope of research:

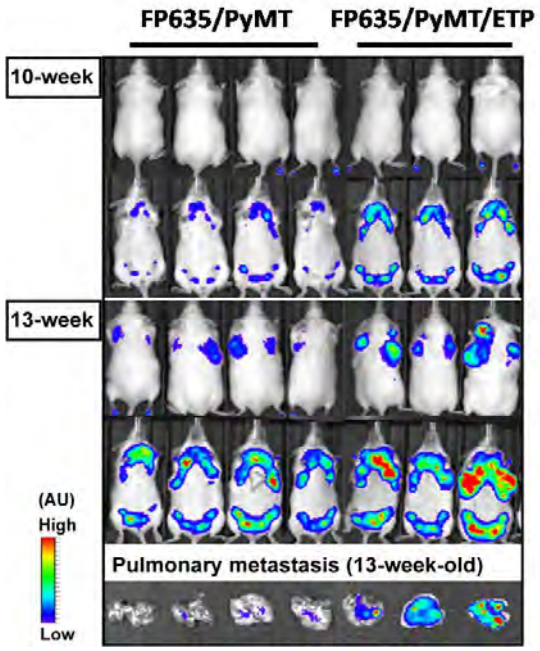
Here, we highlight the adipocyte-derived extracellular matrix as an important and novel site of modulation of cancer cell behavior within the tumor microenvironment. Contributions of stromal adipocytes on cancer cell behavior are expected to have more relevance to obesity-related cancers such as colon, renal, pancreas, and post-menopausal breast cancer. Our studies will highlight novel mechanisms linking obesity and aggressive cancer progression and provide therapeutic strategies for these obesity-related cancers.

BODY

***Specific Aim1*. Analyze the phenotype of MMTV-COL6A3-C5 transgenic mouse in the background of MMTV-PyMT breast cancer model.**

Task 1. Determine the pro-mitotic activities of COL6A3-C5 in mammary cancer cells (1-9 months): ***Completed in year 1***
Task 2. Characterization of the VIa3-C5 transgenic mouse model in the MMTV-PyMT background (1-24months): ***Completed in year 1***

Development of in vivo imaging tools to quantify tumor volume:



The detailed quantification of lesion size remains a challenge. If the differences in lesion growth are visually apparent, the appropriate quantification is fairly straightforward. For cases in which the differences are more subtle, I am currently establishing new protocols for volume integration of lesions using an infrared scanner. I have generated the MMTV-infrared fluorescence transgenic mice, which will allow us to visualize breast tumor progression in whole animals with the IVIS scanner (UTSW Cancer Imaging Center). We bred MMTV-F635 mice with COL6A3-C5 mice to quantify tumor volume at the whole animal level (**Fig. 1**).

Figure 1. **Representative whole body image for tumor burden. Fluorescence protein (FP635) expressing transgenic mice driven by MMTV promoter was introduced into PyMT and PyMT/COL6A3-C5 mice.** Tumor volume for 10- and 13-week-old FP635/PyMT and FP635/PyMT/ETP (COL6A3-C5) mice was monitored by fluorescence scanner (IVIS, Caliper life science). Metastatic burden was determined by fluorescence signals in lung tissues.

***Specific Aim2*. Generation of polyclonal antibodies and monoclonal antibodies that can neutralize the activity of the collagen VIα3-C5 domain, the region of the protein that we believe to confer resistance to cisplatinum through induction of metallothioneins**

Task 1. Generate VIa3-C5 specific polyclonal antibodies (1-12 months): ***completed in year 1***
Task 2. Generation of VIa3-C5 specific monoclonal antibodies (1-12 months): ***completed in year 1***
Task 3. Verify the neutralizing activities of VIα3-C5 monoclonal antibodies (1-12 months): ***completed in year 1***

***Specific Aim3*. Compare the susceptibility to platinum-based cancer regimens in wild-type vs. collagen VI null mice ($COL6^{-/-}$) in the MMTV-PyMT (6-24 months).**

Task 1. Generate collagen VI null mice and breed with MMTV-PyMT mice (6-12 months): ***Completed in year 1***

Task 2. Characterize the collagen VI effects on cisplatin resistance in MMTV-PyMT mice model (9-24 months): ***ongoing***

2a. Evaluate cisplatin drug toxicity and set up the optimal protocol of cisplatin treatment in wild-type and collagen VI null mice in the MMTV-PyMT background (9-18 months): ***Completed***

We have tested various ranges of cisplatin dosages from 1 to 2.5 mg/kg. We decided to apply either 2.5 mg/kg or 1 mg/kg by intraperitoneal injection (I.P) three times per week for cisplatin mono-therapy or cisplatin and thiazolidinedione (TZD) combination therapy. We limit the cisplatin treatment to no more than 2-months because of drug toxicity.

In this experimental paradigm, we show that primary tumor growth in PyMT/COL6$^{-/-}$ mice is significantly attenuated after cisplatin treatment compared to PyMT/COL6$^{+/+}$, suggesting that adipocyte derived COL6 affects cisplatin susceptibility in mammary cancer (ref. Figure 1 of annual report for the first year). However, we realized that the current experimental setting has a potential drawback. The rate of tumor growth in PyMT/COL6$^{-/-}$ is significantly attenuated, even in the cisplatin free groups, due to the fact that COL6 affects tumor growth itself. Thus, we compared the cisplatin resistance with size-adjusted tumors from PyMT/COL6$^{-/-}$ and PyMT/COL6$^{+/+}$ mice. We have consistently observed that PyMT/COL6$^{-/-}$ mice are ***more sensitive*** to cisplatin treatment compared to tumor-size matched PyMT/COL6$^{+/+}$ mice. The differences however fail to reach statistical significance (**Figure 2**).

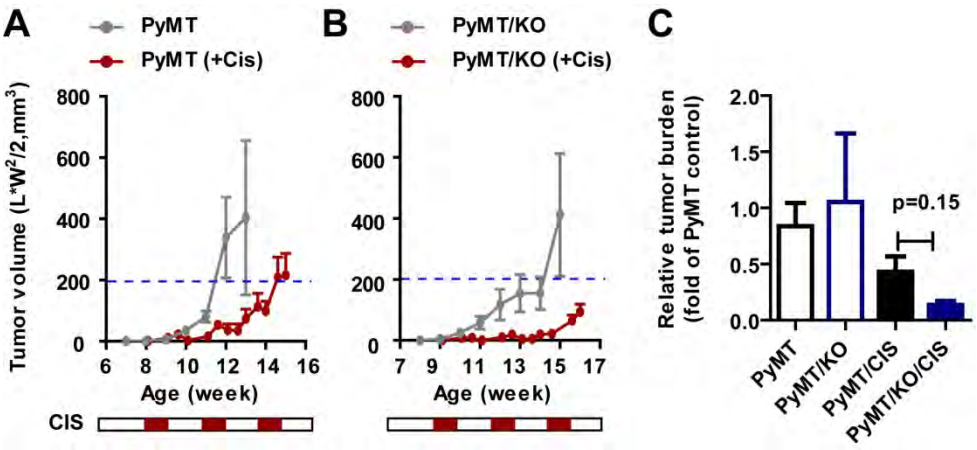


Figure 2. PyMT/COL6$^{-/-}$ mice are more susceptible to cisplatin treatment. A. 8-week-old PyMT/COL6$^{+/+}$ vs. **B**. 9-week-old PyMT/COL6$^{-/-}$ mice were treated with 1 mg/kg cisplatin by intraperitoneal injection (i.p.) two times a week. Tumor progression was measured by caliper during cisplatin treatments. n=10 for cisplatin^{-} and n=10 for cisplatin^{+} group. **C**. Quantitative results are represented by Mean ± SEM. p=0.15 vs. PyMT (+cisplatin) by *unpaired t-test*.

2b. Tumor tissues or MET cells (mammary epithelial tumor cells) from PyMT/COL6$^{+/+}$ vs. PyMT/COL6$^{-/-}$ mice will be implanted into WT mice. Cisplatin treatment will be performed on the tumor bearing mice when the tumor size reaches 5 mm diameter and treatment will be ceased when the tumor regression is apparent. (12-24 months): ***ongoing***

Tumor progression in MMTV-PyMT mice is very aggressive. They develop early carcinomas to late carcinomas by 10-weeks and 13weeks of age, respectively. This is a very narrow time frame to determine drug resistance. To resolve these problems, we will test cisplatin resistance with the FVB wild-type mice after implantation of primary cultured mammary epithelial tumor cells (MET cells) from tumor tissues of PyMT/COL6$^{+/+}$ vs. PyMT/COL6$^{-/-}$ mice. I will start cisplatin treatment when the tumor size reaches 5 mm diameter and stop treatment when the tumor is regressed upon cisplatin exposure. This treatment protocol will be repeated until they show cisplatin resistance to determine whether or not COL6 affects cisplatin susceptibility in this setting. Our alternative experimental setting will allow us to investigate the cisplatin resistance with more accuracy and longer time frames of tumor latency.

2c. Treat experimental cohorts with cisplatin and measure tumor volumes, rates of apoptosis, and expression levels of metallothioneins (MTs) (18-24 months). To obtain quantitative results with significance, I will perform these experiments in triplicates using independent cohorts: ***completed***

We assessed tumor tissues from PyMT/COL6$^{-/-}$ with or without cisplatin treatment by histological analysis. H&E stained tumor tissues from both groups clearly indicate that COL6$^{-/-}$ tumors are more susceptible to cisplatin treatment compared to COL6$^{+/+}$ tumors (**Figure 3A**). Furthermore, tumor cell apoptosis was significantly increased in PyMT/COL6$^{-/-}$ mice by cisplatin treatment compared to PyMT/COL6$^{+/+}$ tumors (**Figure 3B-C**).

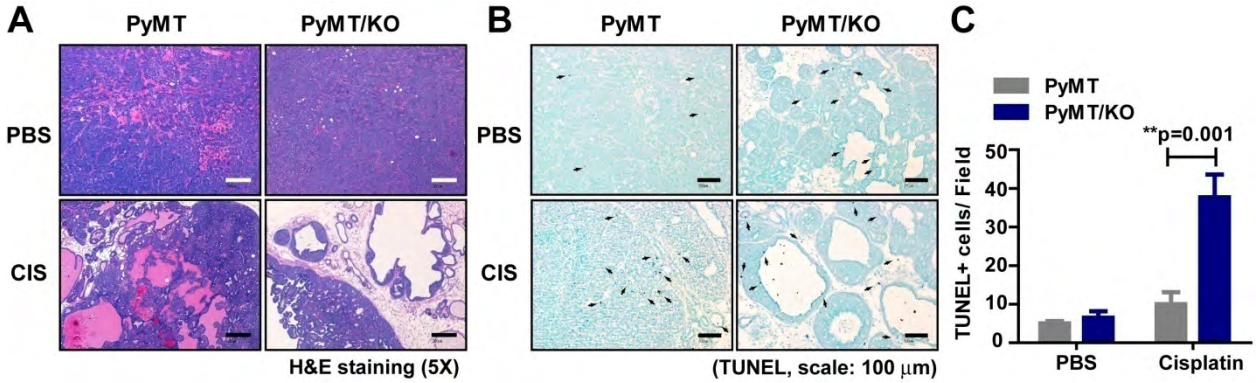
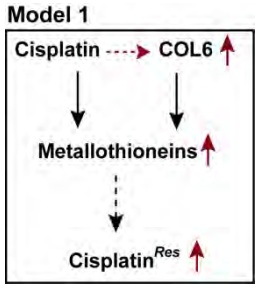


Figure 3. Histological analysis of tumor tissues. A. H&E staining. Scale bar: 200 μm. **B.** Apoptosis is determined by TUNEL assay. Arrows indicated TUNEL positive cells. Scale bar: 100 μm. **C**. Quantitative results for TUNEL assay are represented by Mean ± SEM (n=5 per each groups). **P=0.001 vs. PyMT (+cisplatin) by *unpaired t-test*.

We hypothesized that increased levels of metallothioneins (MTs) are induced by COL6 which, may in turn cause drug resistance during cisplatin treatment (**Model 1**).



To see whether the levels of metallothioneins (MTs) are down-regulated in tumor tissues from PyMT/COL6$^{-/-}$, we determined protein and mRNA levels for MTs-1, -2, -3, and -4 by immunohistochemistry and quantitative-RT-PCR, respectively (**Figure 4**). Both protein levels (**Figure 4A-B**) as well as mRNA levels for MTs were significantly decreased in tumor tissues from PyMT/COL6$^{-/-}$ after cisplatin treatment compared to control tumor tissues, which show high levels of MTs in tumor tissues upon cisplatin exposure (**Figure 4C**). These results suggest that **COL6 is responsible for the cisplatin-induced increase of MTs (MT-1 through MT-4) levels associated with drug resistance.**

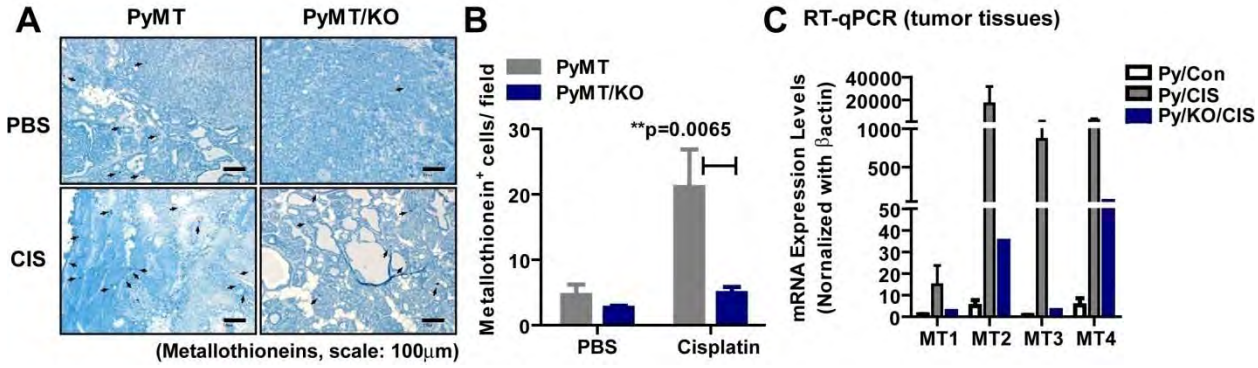


Figure 4. Metallothionein levels are significantly decreased in tumor tissues from PyMT/COL6$^{-/-}$ mice compared to PyMT/COL6$^{+/+}$ mice after cisplatin treatment. A. Immunohistochemistry for methallothioneins. Arrows indicate metallothioneins positive cells. **B.** Quantitative results for MTs are represented by Mean ± SEM. **P=0.0065 vs. PyMT (+cisplatin) by *unpaired t-test*. (n=5 per each groups) **C**. mRNA levels for MT-1, -2, -3, and -4 were determined by RT-qPCR with tumor tissues. Values were normalized with β-actin and represented by Mean ± SEM (n=7-10 per each groups).

Specific Aim4. **Examine the roles of collagen VI on synergistic effects between thiazolidinediones (TZDs) and cisplatin therapy (3-36 months)**

Task 1. Examine the regulation of VIα3-C5 by TZDs treatment *in vivo* (3-8 months): ***ongoing***
1a. Obtain experimental cohorts (3-6 months): At least 5 mice will be used in the experiments.
1b. Perform immunohistochemistry (IHC) with histology samples of mammary gland tissues of MMTV-PyMT mice with or without TZD treatments for 2 to 4 weeks (4-8 months): slides are ready for these experiments: ***ongoing***

Task 2. Compare cisplatin susceptibility in collagen VI null and wild type mice in the background of MMTV-PyMT mice with or without TZDs treatments (6-24 months): ***ongoing***
2a. Get experiment cohorts (6-12 months): 7-10 mice in each group will be used in the experiments.
2b. Optimize the protocols of cisplatin treatment in combination with TZD as mentioned in Specific Aim3- Task 2 (12-18 months)
2c. Perform cohort experiments (18-24 months): Collect samples and analyze it.

We investigated cisplatin susceptibility with or without TZD pre-treatment in PyMT/COL6$^{-/-}$ compared to tumor-size matched PyMT/COL6$^{+/+}$ mice. The results indicate that tumor growth is attenuated in the PyMT/COL6$^{-/-}$ by cisplatin monotherapy (**Figure 2**) or in PyMT/COL6$^{+/+}$ with combination therapy of cisplatin with TZD (**Figure 5B**) compared to PyMT/COL6$^{+/+}$ with cisplatin monotherapy (**Figure 5A**). **This suggests that either COL6KO or a combination therapy with TZD enhance the cisplatin susceptibility in the MMTV-PyMT breast cancer model.** However, synergistic effects of TZD in cisplatin treatment were not observed in PyMT/COL6$^{-/-}$ mice (**Figure 5C**). These results suggest that there is a differential regulatory mechanism in COL6$^{-/-}$ mice in response to TZD compared to wild-type mice after cisplatin treatment, which needs to be investigated further.

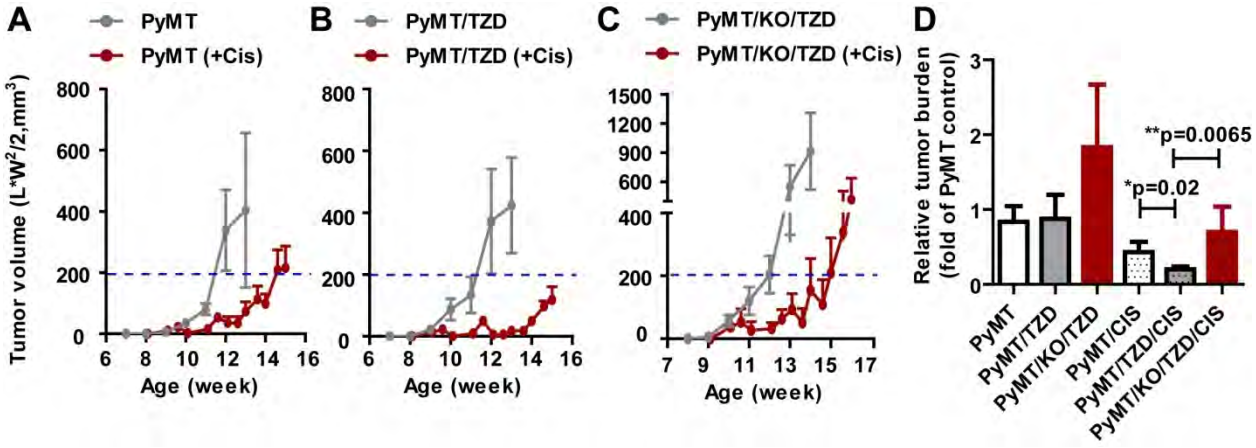


Figure 5. Comparison of cisplatin susceptibility between PyMT/COL6$^{-/-}$ and PyMT/COL6$^{+/+}$ mice in combination with TZD treatment. Rosiglitazone (20 mg/kg/day) was used for 10 days before cisplatin treatment by inclusion in the diet and cisplatin (1 mg/kg) was injected by i.p. twice a week. Tumor volume was determined by caliper measurements during cisplatin treatment from 10 weeks to 17 weeks. The graph represents Mean ± SEM. **A.** PyMT/COL6$^{+/+}$ were treated cisplatin, **B.** PyMT/COL6$^{+/+}$ were treated cisplatin in combination with TZD, **C.** PyMT/COL6$^{-/-}$ were treated cisplatin in combination with TZD. **D**. Tumor volume at the time of end of study, 17 weeks were quantified and represented by Mean ± SEM. *P=0.02 vs. PyMT (+cisplatin); **p=0.0065 vs. PyMT (+TZD and cisplatin) by *unpaired t-test*. n= 7-10 mice per each group.

We analyzed tumor tissues by immunohistochemistry and quantitative RT-PCR. The necrotic lesion area was significantly increased in tumors pre-treated with TZD (PyMT/TZD/CIS) compared to control (PyMT/CIS) whereas these effects were abolished in the COL6$^{-/-}$ mice treated with TZD (PyMT/KO/TZD/CIS) as determined by H&E staining (**Figure 6A**). Tumor cell apoptosis was

comparable between PyMT/TZD and PyMT/KO/TZD by cisplatin treatment suggesting that the **combination therapy of cisplatin with TZD increased tumor cell apoptosis in both PyMT/COL6$^{+/+}$ and PyMT/COL6$^{-/-}$ mice compared to cisplatin monotherapy in PyMT mice** (**Figure 6B-C**). Thus, the degree of tumor cell apoptosis could not account for a difference of tumor growth between PyMT/TZD and PyMT/KO/TZD group. We will investigate tumor cell proliferation or survival to see if these are associated with increased tumor growth in PyMT/KO/TZD group compared to either PyMT or PyMT/TZD group.

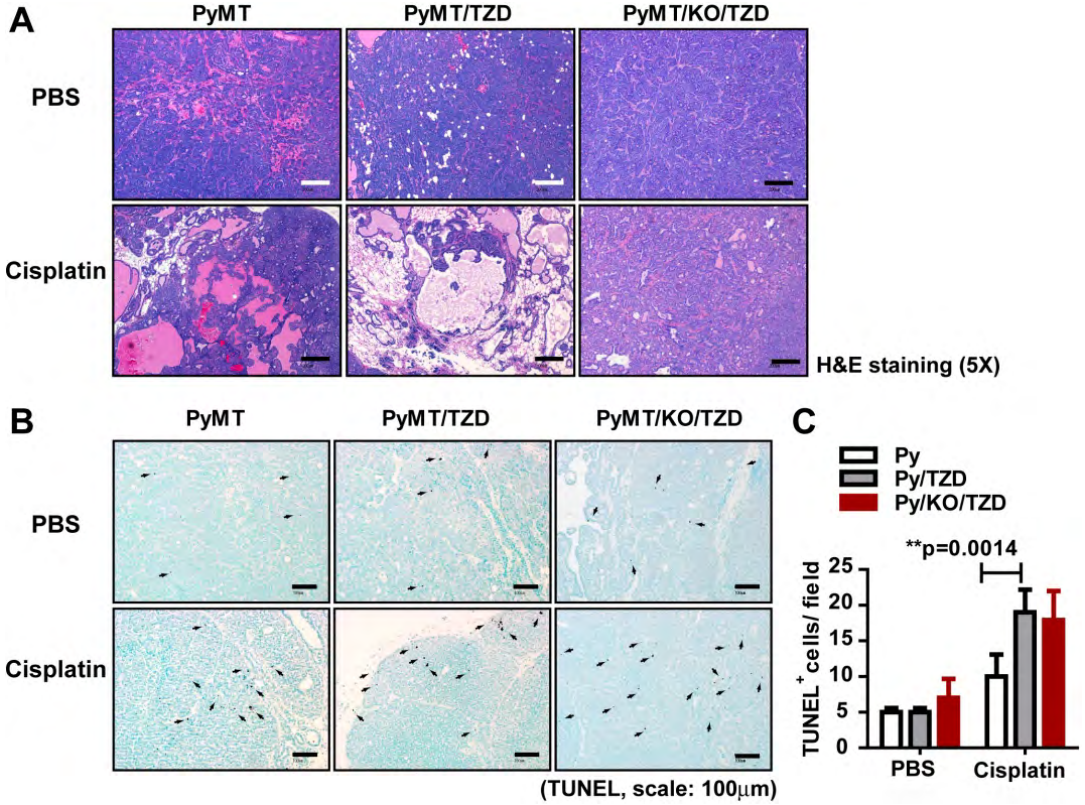


Figure 6. Histological analysis for tumor tissues. A. H&E staining. Scale bar: 200 μm. **B.** Apoptosis is determined by TUNEL assay. Arrows indicate TUNEL positive cells. Scale bar: 100 μm. **C**. Quantitative results are represented by Mean ± SEM. **P=0.0014 vs. PyMT (+cisplatin) by *unpaired t-test*. n=5 mice per each group.

Model 2

PyMT/WT	PyMT/COL6A1KO
TZD + CIS → COL6 ↓	TZD + CIS → COL6A3 ↑
↓ ↓	↓ ↓
Metallothioneins ↓	Metallothioneins ↑
⇣	⇣
Cisplatin^{Res} ↓	Cisplatin^{Res} ↑

We found that TZD conveys a synergistic effect on cisplatin susceptibility, which is diminished by COL6A1KO mice in the background of MMTV-PyMT mice. First, we hypothesized that a synergistic effect of TZD in combination with cisplatin is mediated by decreased levels of COL6, which is in accordance with the previous results that COL6KO mice enhanced cisplatin susceptibility (**Figure 2-4, and Model 1**). To test this (see **Model 2-PyMT/WT)**, we determined the mRNA levels for MTs and COL6 to see whether TZD-mediated decrease in COL6 is associated with decreased MTs levels. mRNA levels for COL6 (A1, -A2, and A3) were slightly decreased by TZD treatment (**Figure 7B**). We will also determine protein levels for COL6A3 by immunohistochemistry as mentioned in SA4-Task1. The mRNA levels for MTs (MT1-MT4) in tumor tissues were significantly increased by cisplatin treatment (**Figure 7A, PyMT vs. PyMT/CIS**) whereas they were attenuated by TZD treatment or by COL6KO (**Figure 7A, PyMT/TZD/CIS, PyMT/KO/CIS vs. PyMT/CIS**). These results suggest that an enhancement of cisplatin susceptibility by TZD is acquired by decreased levels of MTs, which may be associated with COL6 levels in tumor tissues. Protein levels for MTs were also determined by immunohistochemistry (**Figure 8**).

Interestingly, the decreased levels of MTs in either TZD-treated or COL6$^{-/-}$ mice were completely reversed by TZD treatment in COL6$^{-/-}$ mice (**Figure 7A, PyMT/KO/TZD/CIS**). To see whether there is a compensatory increase of other types of collagens in COL6A1$^{-/-}$ mice after TZD treatment with cisplatin, we determined mRNA levels for various types of collagens (**Figure 7B**).

Particularly, we found that COL6A3 is highly up-regulated in COL6A1$^{-/-}$ mice by TZD treatment with cisplatin (PyMT/KO/TZD/CIS) compared to the TZD control group (PyMT/TZD/CIS, **Figure 7B**) which is consistent with the levels of MTs. These results indicate that there is a compensatory increase of other forms of COL6, in particular the COL6A3 subunit in COL6A1$^{-/-}$ mice. This probably accounts for the increase in tumor growth. Based on our previous results in COL6A3 transgenic mice, the C5 domain of COL6A3 plays a crucial role in tumor growth and metastasis (**Figure 1**). The unexpected increase of COL6A3 in COL6A1$^{-/-}$ mice after cisplatin treatment with TZD may be part of the mechanism explaining abnormal tumor growth in COL6A1$^{-/-}$ mice during combination therapy of cisplatin with TZD (**Model 2-PyMT/COL6A1KO**). To pursue further whether COL6A3 is associated with cisplatin resistance, we will investigate cisplatin resistance with our COL6A3-C5 transgenic mouse model which I have generated.

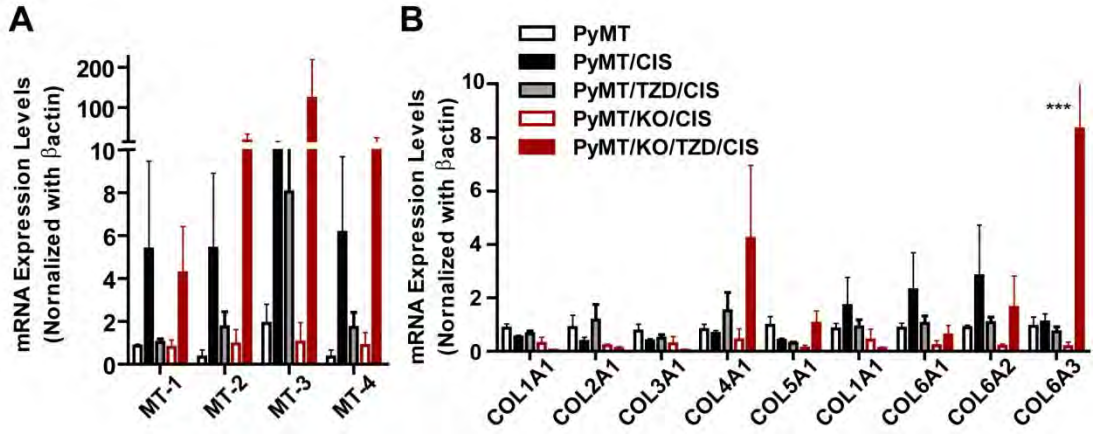


Figure 7. mRNA levels for metallothioneins and various types of collagens in response to TZD in PyMT/COL6$^{+/+}$ or PyMT/COL6$^{-/-}$ mice after cisplatin treatment. Tumor tissues were analyzed for mRNA levels by quantitative RT-PCR. **A.** mRNA levels for MT-1, -2, -3, and -4 were represented by Mean ± SEM after normalized with β-actin. **B**. mRNAs for various types of collagens were analyzed by qRT-PCR. Results were represented by Mean ± SEM after normalized with β-actin. ***p<0.001 vs. PyMT/CIS by *2-way ANOVA*. n= 7-10 mice per each group.

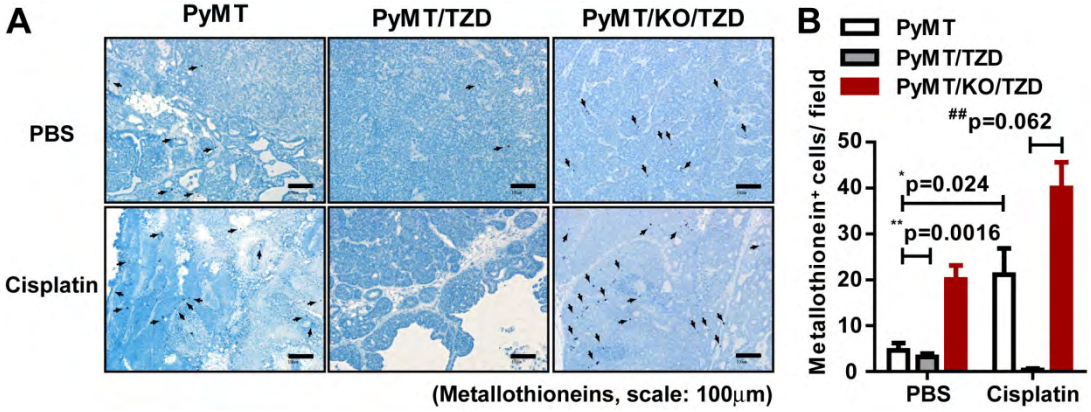


Figure 8. Immunohistochemistry for Metallothioneins levels. A. Tumor tissues were immunostained with MT antibodies. Arrows indicate metallothioneins positive cells. **B.** Quantitative results are represented by Mean ± SEM. **p=0.0016 vs. PyMT (PBS); *p=0.024 vs. PyMT (PBS); $^{\#\#}$p=0.062 vs. PyMT/TZD (+cisplatin) by *unpaired t-test*. n=7-10 mice per each group.

Task 3. Characterize the effects of VIα3-C5 neutralizing monoclonal antibodies in combination with TZDs to demonstrate synergistic enhancement of susceptibility to cisplatin treatment for breast cancer (18-36 months): ***on-going***

3a. Tumor tissues or MET cells (mammary epithelial cells) from PyMT/COL6$^{+/+}$ mice will be implanted into WT mice.

3b. Perform the treatment with TZD and VIα3-C5 monoclonal antibodies in combination with cisplatin

3c. Collect samples and analyze the tumor progression and cisplatin susceptibility (24-36 months).

***Specific Aim5*. Verify the relevance of VIα3-C5 levels in clinical samples (6-36 months).**

Task 1. Verify the levels of VIα3-C5 in breast cancer mouse subjects (6-12 months).

1a. Measure the VIα3-C5 protein levels in MMTV-PyMT mice: ***completed in first year***

1b. Measure the circulating levels of VIα3-C5 (6-12 months): Development of ELISA method to detect circulating VIa3-C5 proteins in the serum samples of mouse and human breast cancer subjects. At least 5 samples in each group will be used in this experiment: ***on-going***

We have been developing the sandwich-ELISA method using COL6A3-C5 specific rat monoclonal antibody and rabbit polyclonal antibody as a capturing and detection antibodies. We expect to have optimal conditions shortly for ELISAs to measure the COL6A3-C5 in circulation. Initially we used an anti-mouse COL6A3-C5 polyclonal Ab which seems to be less efficient to detect human C5. Thus, we have generated a novel **anti-human C5** rabbit polyclonal antibody which is specific for human (**Figure 9**). We will set up the optimal conditions for the ELISA measuring human C5 levels in circulation.

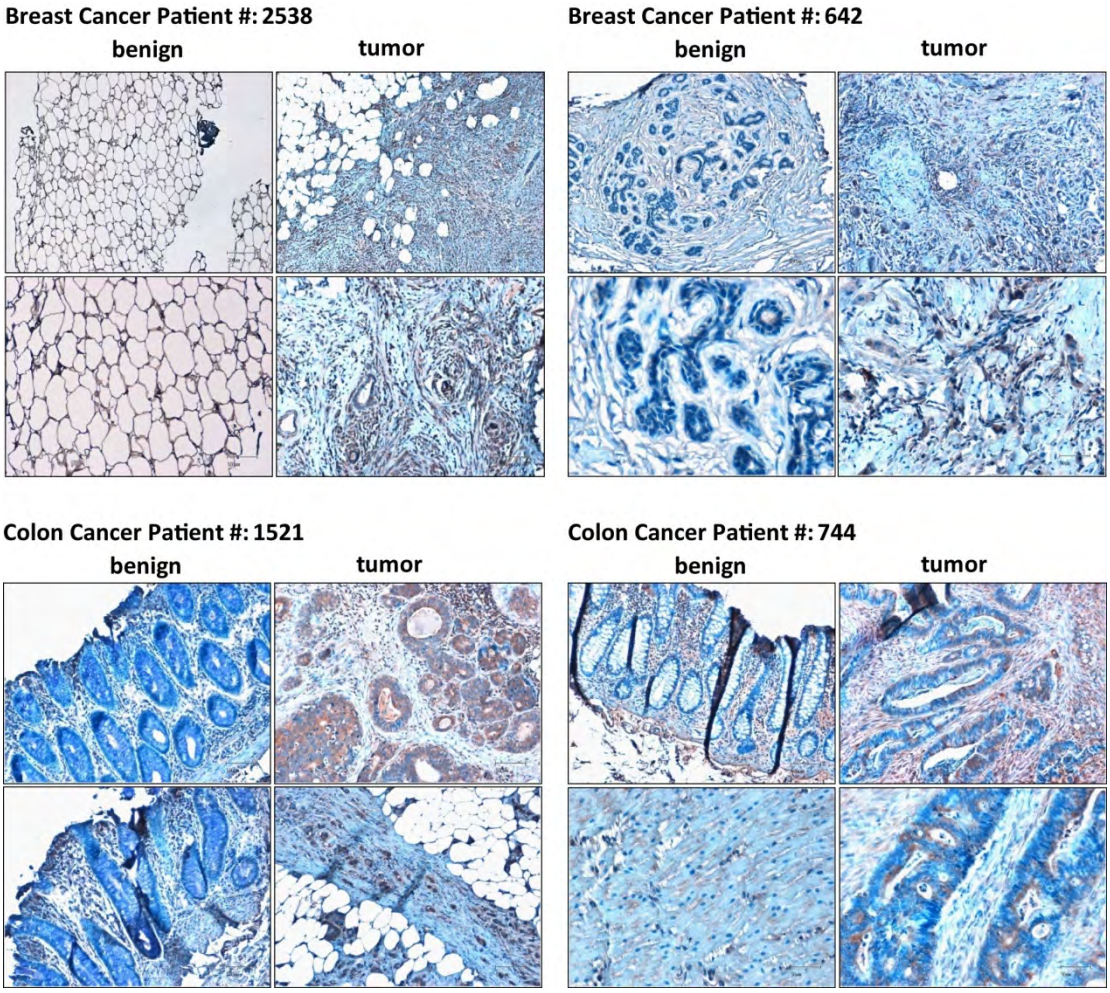


Figure 9. immunohistochemistry for human COL6A3-C5. De-identified human patient samples were obtained from the human tissue bank (UTSW Medical Center) and assessed for COL6A3-C5 levels by immunostaining with anti-human COL6A3-C5 specific polyclonal antibody (TX933). Scale bar is represented in the images (100 μm or 50μm).

Task 2. Verify the levels of VIα3-C5 in breast cancer human subjects (12-36 months): *on-going*

2a. Collect human tissue samples from UTSW cancer tissue bank and analyze the levels of VIα3-C5 by immunohistochemistry using VIa3-C5 antibodies (12-18 months): ***completed***

We have determined COL6A3-C5 levels in human breast cancer patients (**Figure 9, breast cancer patients**) and colon cancer patients (**Figure 9, colon cancer patients**) with anti-human C5 polyclonal antibody. **C5 is highly expressed in both types of tumor tissues compared to benign tissues. Thus, COL6A3-C5 is a promising cancer biomarker not only for breast cancer but also other tumors, such as colon cancers**.

2b. Measure the VIα3-C5 levels in both serum and breast tumor samples of breast cancer patients at UTSW Medical Center (24-36 months): ***on-going***

As mentioned in SA5-Task1, we will optimize an ELISA system to measure COL6A3-C5 levels in human serum samples.

2c. Measure the VIα3-C5 levels in human samples after platinum-based chemotherapy in combination with TZDs to characterize VIα3-C5 effects on drug resistance (24-36 months).

Our collaborator Dr. David Euhus at UTSW Medical Center will play a critical role in spearheading and facilitating these efforts.

KEY RESEARCH ACCOMPLISHMENTS

Year 1

- **Established COL6A3-C5 transgenic mice under the control of MMTV promoter: low and high expressors identified.**
- **Generated both polyclonal and monoclonal antibodies against COL6A3-C5 domain.**
- **Determined the COL6A3-C5 protein levels in both human and mouse mammary tumor tissues by immunohistochemistry with COL6A3-C5 specific polyclonal antibodies.**
- **Determined anti-apoptotic and pro-mitotic activities of COL6A3-C5 protein *in vivo.***
- **Determined the ratio of tumor progression and pulmonary metastasis in COL6A3-C5 transgenic mice in the background of MMTV-PyMT (PyMT/COL6A3-C5) compared to control littermates *in vivo.***
- **cDNA microarray analysis successfully performed with tumor tissues from PyMT/COL6A3-C5 and PyMT mice.**
- **Generation of MMTV-F635 (infrared fluorescence protein) transgenic mice for *in vivo* imaging of mammary tumor progression successfully accomplished.**
- **Optimized the protocols for immunotherapy with COL6A3-C5 monoclonal antibodies.**
- **Investigated the cisplatin susceptibility in combination with TZD to examine the effects of COL6 on drug resistance (large cohort experiments).**

Year 2

- **Determined the tumor burden at the whole body level with the MMTV-FP635 transgenic mice through *in vivo* imaging.**
- **Determined the cisplatin susceptibility of PyMT/COL6$^{-/-}$ mice compared to tumor-size matched PyMT/COL6$^{+/+}$ mice.**
- **Determined the cisplatin susceptibility in combination with TZD to examine the effects of COL6 on drug resistance (large cohort experiments).**
- **Established the protocols for primary culture of cancer cells originated from MMTV-PyMT tumor tissues.**
- **Introduced cancer cell implantation methods into cisplatin treatment protocols in combination with TZD to examine the effects of COL6 on drug resistance.**
- **Generated a novel anti-human COL6A3-C5 rabbit polyclonal antibody, which is more specific for human C5 as determined by immunohistochemistry.**

REPORTABLE OUTCOMES

Reportable outcomes that have resulted from this research project:

- Manuscripts:
  - *in preparation*
- Abstracts:
  - 2011 UKC Conference, Park City, Utah.
- Oral Presentations:
  - Touchstone Diabetes Center Seminar Series at UTSW.
  - 2011 Era of Hope Conference, concurrent symposium session, Orlando, Florida.

CONCLUSION

We have characterized the properties of the adipocyte-derived type 6 collagen-alpha3-C5 domain (COL6A3-C5) on mammary tumor progression and metastasis *in vivo*. We established transgenic mice that express COL6A3-C5 under the control of the MMTV promoter. We bred these mice with MMTV-PyMT mice to investigate COL6A3-C5 effects on mammary tumor progression and metastasis. Our results indicate that **COL6A3-C5 is a potent new mitogen**, **stimulating tumor cell proliferation** as well as **enhancing pulmonary metastasis *in vivo***. We will pursue these observations further to quantify the properties of cancer cells with respect to motility and invasion regarding metastasis with primary cultured mammary epithelial tumor cells (MET cells) from PyMT/COL6A3-C5 and PyMT mice. To identify the molecular mechanisms on COL6A3-C5 mediated cell proliferation and survival, we performed cDNA microarray analysis with tumor tissues from 12-week old PyMT/COL6A3-C5 and PyMT mice. Based on our microarray results, 45 % of genes significantly modulated by COL6A3-C5 are related to phosphorylation, suggesting that COL6A3-C5 is a signaling molecule stimulating a kinase (or a phosphatase) activity. We currently analyze the microarray data in further detail and will focus on several target molecules regulated by C5-mediated signaling pathways.

For the therapeutic purposes, we generated COL6A3-C5 specific monoclonal antibodies and have tested these on a mouse mammary tumor model, the MMTV-PyMT mice. Our preliminary results suggest that COL6A3-C5 monoclonal antibodies **have potent protective effects on mammary tumor progression**. We will pursue to determine the efficacy of COL6A3-C5 monoclonal antibodies more carefully.

We have investigated cisplatin resistance of COL6 knock-out mice in the background of MMTV-PyMT mice and compared that to control MMTV-PyMT mice. PyMT/COL6$^{-/-}$ mice were more susceptible to cisplatin treatment relative to PyMT as judged by tumor volume over the course of cisplatin treatment. However, we noticed that it is challenge to determine the drug resistance of MMTV-PyMT mice, because the tumor growth in this model is so aggressive. To address these questions under better conditions, we will use tumor cell implants with primary cultured mammary epithelial tumor cells from PyMT/COL6$^{-/-}$ vs. PyMT mice. This will allow us to determine cisplatin resistance over a longer time period of tumor progression.

Finally, I will measure COL6A3-C5 levels in human cancer patient serum samples with the ELISA which we are currently optimizing for the conditions with monoclonal- and polyclonal antibodies against COL6A3-C5. If I can detect the COL6A3-C5 level in human samples, this has the potential to be a useful therapeutic marker for cancer patients as outlined in our original rationale.

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