

## Molecular Pathology of Tumor Progression

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Tumor progression is the term for the gradual cellular and molecular changes that occur during the development of tumors. We are studying the role of pro-protein convertases (PCs) such as PACE-4 and furin during the late stages of tumor progression (i.e., cancer cell invasion and metastasis). Overexpression of PCs correlates with an aggressive neoplastic phenotype both in mouse models and in human primary tumors. Genetic transfer of PACE-4 or furin cDNA into non-tumorigenic cell lines and low-grade squamous cell carcinoma cell lines induced invasiveness and tumorigenicity. Inhibition of these effects can be obtained by transfecting either the bioengineered serpin alpha1-PDX or the propeptide of the furin PC. Similar effects were obtained with the drug dec-RVKR-chloro-methyl-ketone (CMK). These inhibitors are able to decrease or abolish the invasive/malignant phenotype of tumor cells by inhibiting the activation of invasion and metastasis-associated gene products such as MT1-MMP, stromelysin-3, TGF- $\beta$  and IGF-1R.



Transgenic mice in which PACE4 was targeted to the epidermis and oral epithelia show increased susceptibility to carcinogenesis associated with a defect in epithelial basement membranes that increases the ability of cancer cells to penetrate into the subepithelial tissues, thus accelerating the process of tumor cell invasion. Another unrelated gene, discovered during differential display together with PCs is VILIP-1, a member of the neuronal Ca<sup>++</sup> sensor protein family. VILIP-1 is able to act as a tumor suppressor in mouse skin squamous carcinoma cells inhibiting cell proliferation, adhesion and invasion by modulating cAMP levels as well as inactivating MMP-9 and RhoA activity. Recently, we have found that this gene may have similar properties in human esophageal and lung tumors.

### **PACE 4 expression in mouse basal keratinocytes results in basement membrane disruptions and acceleration of tumor progression.**

Bassi, Cenna, Klein-Szanto, in collaboration with Lopez<sup>§</sup>

Collagen type IV degradation results in disruption and breakdown of the normal basement membrane (BM) architecture, a key process in the initiation of tumor microinvasion into the connective tissue. PACE4, a proprotein convertase, activates membrane type metalloproteinases (MT-MMPs) that in turn process collagenase type IV. Because PACE4 is overexpressed in skin carcinomas and *in vitro* overexpression of PACE4 resulted in enhanced invasiveness, we investigated whether *in vivo*

PACE4 expression leads to the acquisition of invasiveness and increased tumorigenesis. Two transgenic mouse lines were designed targeting expression of PACE4 to the epidermal basal keratinocytes. Transgenic keratinocytes showed increased processing of MT1-MMP and MT2-MMP resulting in collagenase IV activation and collagen type IV degradation. Higher collagenolytic activity partially disrupted normal basement membrane architecture favoring epithelial endophytic growth toward the dermis and accelerating invasion and metastasis after chemical carcinogenesis. PACE4 overexpression resulted in enhanced susceptibility to carcinogenesis and tumor progression pointing to a new target for blocking tumor cell invasiveness.

### **Different proprotein convertase pattern of expression in ovarian tumor progression.**

Bassi, Al-Jumaily, Zhang, Cenna, in collaboration with Page,<sup>§</sup> Schilder,<sup>§</sup> Godwin<sup>§</sup>

Proprotein convertases (PCs) is a family of serin proteases that activate many substrates that are crucial for tumor progression. Four of the PCs, furin, PC-7, PACE-4 and PC-5 are ubiquitously expressed. Although they accomplish similar biochemical functions, i.e., proteolytic cleavage at the C-terminal portion of the RXR/KR motif, their different subcellular location as well as subtle substrate preferences suggests that their activities are not redundant.

In order to determine if the pattern of expression of each of these proteases differ during ovarian tumor progression, we evaluated the expression of furin, PC-7, PACE-4 and PC-5 in human normal and cancer cell lines and in primary tumors. We found that furin RNA expression, determined by RT-PCR, was predominant in cancer cell lines. In addition, its expression was strictly associated with less than five-year survival. Protein analysis by Western blot of cell lysates or immunohistochemistry of tumors indicated that furin expression was higher in cancer cell lines or tissues than in their normal counterparts. Conversely, we observed PC-7 RNA expression mainly in normal cell lines and in tumors from patients surviving for more than five-years after diagnosis. As in the case of PC-7, we observed PACE-4 silencing in cancer cell lines and, less pronounced in tumors. PC-5 did not show significant expression in most of the samples analyzed. The differential expression in normal and cancer cells/tissues suggests that the ubiquitously expressed PCs may play different roles in ovarian tumor progression, supporting the hypothesis that the activities of the various PCs are not redundant. We conclude that these PCs follow a distinct pattern of expression during ovarian tumor progression. Furthermore, furin is associated with poor prognosis.

**Visinin-like protein-1 is a potent inhibitor of cell adhesion and migration in squamous carcinoma cells.** Klein-Szanto, in collaboration Gonzalez Guerrero,<sup>§</sup> Jaffer,<sup>§</sup> Page,<sup>§</sup> Braunewell,<sup>a</sup> Chernoff<sup>§</sup>

Tumor cell invasion is a highly integrated and complex process constituting one of the essential components of tumor progression. We have

previously reported that Visinin-like protein-1 (VILIP-1), a member of the neuronal EF-hand calcium-sensor protein family, plays an important role in regulating tumor cell invasiveness of mouse squamous cell carcinoma (SCC). Previously we have shown VILIP-1 enhanced cAMP levels through PKA induction. However, the mechanism by which VILIP-1 reduces the invasive capacity of SCC cells is not well understood. The aim of this study is to determine whether VILIP-1 promotes changes in cell adhesion and migration of SCC cells.

Overexpression of VILIP-1 reduced murine cell adhesion to fibronectin. VILIP-1 overexpression also led to decreased cell migration together with downregulation of  $\alpha$ v and  $\alpha$ 5 integrin subunits. Conversely, knocking-down VILIP-1 protein by short hairpin RNA, in low-grade SCC cells, resulted in increased cell motility. These results point to a critical role of VILIP-1 in regulating cell adhesion and migration. Decreased or absent VILIP-1 expression in SCC cell subpopulations would lead to a more advanced malignant phenotype characterized by changes in adhesive ability and increased cell motility due to changes in integrin expression, suggestive of a tumor suppressor function.

**VILIP-1, a type two tumor suppressor gene, is downregulated in human lung cancer.** Fu, Fong, Cenna, Bassi, Klein-Szanto, in collaboration with Apostolou,<sup>§</sup> Bellacosa<sup>§</sup>

VILIP-1, a member of the neuronal Ca<sup>++</sup> sensor protein family, is able to act as a tumor suppressor in skin squamous carcinoma cells inhibiting cell proliferation, adhesion and invasion by modulating cAMP levels as well as inactivating MMP-9 and RhoA activity. To further study VILIP-1 in other tumor types, we characterized its expression pattern by Western Blot analysis of the NCI 60 panel of tumor cells. We found that VILIP1 gene expression was undetectable in several types of tumor cells, e.g., non-small cell lung carcinoma (NSCLC), breast and ovarian carcinoma lines. In 10 out of 11 NSCLC cell lines, VILIP-1 was not detected, while its expression was present in normal human bronchial epithelial cells. Consistent with this, immunohistochemistry of 42 primary NSCLC showed that 50% of the tumors lacked expression of this protein. No rearrangement or large gene deletion was found in the genomic DNA of selected tumor cell lines.

Neither was the loss of VILIP-1 expression caused by mutations in VILIP-1 proximal 2 kb promoter, exons and exon-intron junction sites. Hypermethylation of the VILIP-1 promoter plays an important role in gene silencing. In most VILIP-1-silent NSCLC cells the VILIP-1 promoter is consistently methylated at certain CpG islands (4/5 cell lines), whereas methylation is not observed in VILIP-1-expressing cells. The VILIP-1 promoter, when chemically methylated *in vitro* before introduction into cells, lost its activity, providing direct evidence that promoter methylation may control VILIP-1 expression. Thus, hypermethylation of the VILIP-1 promoter in NSCLC cells may account for most of the expression changes seen in these cells.

**Bioengineered mouse models show the differential impact of prostaglandin H synthase 1 knockdown on platelets and parturition.** Klein-Szanto, in collaboration with Yu,<sup>b</sup> FitzGerald,<sup>b</sup> Cheng,<sup>b</sup> Fan,<sup>b</sup> Chen,<sup>b</sup> Funk<sup>b</sup>

Platelet activation is a hallmark of severe preeclampsia, and platelet PGH synthase 1-derived (PGHS1-derived) thromboxane A<sub>2</sub> (TxA<sub>2</sub>) has been implicated in its pathogenesis. However, genetic disruption of PGHS1 delays parturition. We created hypomorphic

PGHS1 (PGHS1(Neo/Neo)) mice, in which the substantial but tissue-dependent variability in the inhibition of PGHS1-derived eicosanoids achieved by low-dose aspirin treatment is mimicked, to assess the relative impact of this strategy on hemostatic and reproductive function. Depression of platelet TxA<sub>2</sub> by 98% in PGHS1(Neo/Neo) mice decreased platelet aggregation and prevented thrombosis. Similarly, depression of macrophage PGE<sub>2</sub> by 75% was associated with selectively impaired inflammatory responses. PGF<sub>2</sub>(α) at 8% WT levels was sufficient to induce coordinated temporal oxytocin receptor (OTR) expression in uterus and normal ovarian luteolysis in PGHS1(Neo/Neo) mice at late gestation. The absence of PGHS1 expression in null mice delayed OTR induction and the programmed decrease of serum progesterone during parturition. Thus, extensive but tissue-dependent variability in PG suppression, as occurs with low-dose aspirin treatment, prevents thrombosis and impairs the inflammatory response but sustains parturition. PGHS1(Neo/Neo) mice provide a model of low-dose aspirin therapy that elucidates how prevention or delay of preeclampsia might be achieved without compromising reproductive function.

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Bucks County Chapter Art Show co-chairs Brenda Lawson and George Bramhall both had paintings featured in the exhibit.