

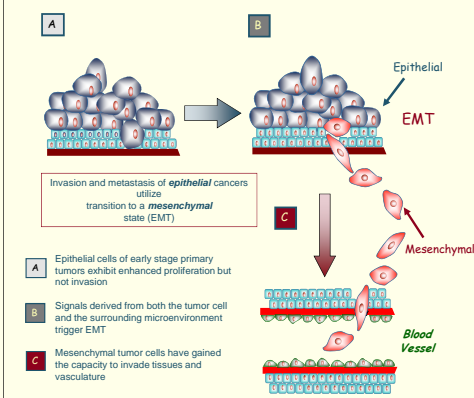
Evaluating Epithelial-to-Mesenchymal Transition (EMT) in the EL1-luc/EL1-SV40 T-antigen transgenic mouse model of pancreatic cancer

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ABSTRACT

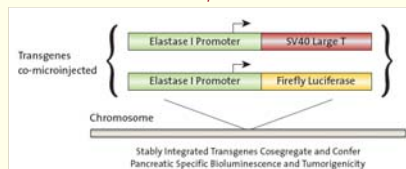
Epithelial-to-mesenchymal transition (EMT) is a biological function important in normal cellular processes such as embryonic development and wound healing. In cancer, it is thought that the tumor cell machinery can re-activate these normal pathways resulting in more aggressive and invasive tumors. The loss of E-cadherin and the gain of vimentin are hallmarks which identify the process of EMT and have been shown to correlate with poor prognosis in multiple solid tumor types. While many preclinical models are utilized to evaluate mechanisms of tumorigenesis few *in vivo* models evaluating parameters of EMT have been described. The EL1-luc/EL1-SV40 T-antigen transgenic mouse represents a model of pancreatic cancer whereby mice develop tissue specific, spontaneous and bioluminescent pancreatic tumors. To evaluate whether EMT occurs in the EL1-luc/EL1-SV40 T-antigen model *in vivo*, we collected primary pancreatic tissue from male mice between 10 and 21 weeks of age. The tissue was formalin fixed, paraffin embedded and then utilized for histopathological endpoints such as Hematoxylin and Eosin staining as well as immunohistochemistry for markers known to be involved in EMT such as E-cadherin and vimentin. We found the tumors to express both markers and become very heterogeneous over time. In early tumors E-cadherin expression is membrane localized and very high. Over time there are areas of the tumors that have reduced or lost E-cadherin expression. Vimentin expression was highly variable but when present tended to be highly expressed. In many of the later stage tumors there was substantial heterogeneity reflected by the appearance of multiple cell types within a tumor. We utilized the Aperio (Aperio Technologies, Vista, CA) slide scanner and software system to evaluate serial sections of tumor samples and found that in some sections of the tumor E-cadherin is present and vimentin is absent, whereas in other areas of the tumor vimentin is present in the absence of E-cadherin. Additionally, we identified areas of the tumor that seem to be expressing both markers which suggests that the EL1-luc/EL1-SV40 T-antigen transgenic mouse may recapitulate many aspects of EMT observed *in vivo*, thus offering a model system to study the signaling and molecular changes necessary for this process during cancer progression.

Introduction: Epithelial-to-Mesenchymal Transition

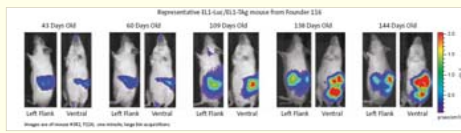


Development of the EL1-luc/EL1-SV40 T-antigen transgenic mouse

Tissue specific transgene strategy to generate bioluminescent mouse model of pancreatic cancer



- > Rat elastase-1 promoter driving tissue specific expression of SV-40 T antigens and F-luciferase
- > Penetration of BL tumor development is 100% by ~60-100 days of age
- > Tumor onset is rapid and ex vivo imaging suggest that independent tumors arise from multiple sites simultaneously
- > Distinct lesions from early hyperplasia to late-stage adenocarcinoma can be identified



Study objective and design

Overall objective:

- > Determine whether EL-1 transgenic mouse tumors undergo EMT *in vivo*

Aim of the studies:

- > Investigate the EMT status of EL-1 pancreatic tumors over time by IHC evaluation of E-cadherin and Vimentin expression

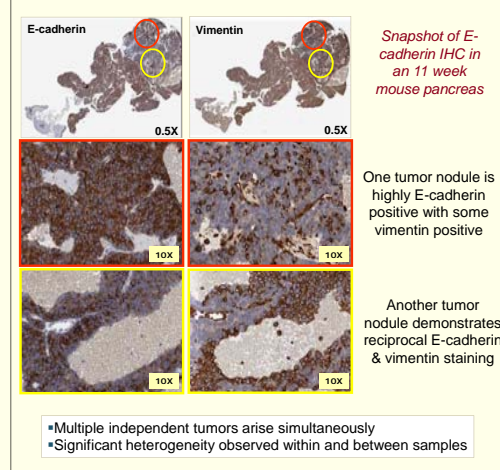
Study Design:

- > Tumor development in male mice was monitored over time by bioluminescent imaging
- > Pancreatic tissue was collected from animals between 11 & 21 weeks of age (n=5/group) for EMT marker analysis
- > Samples were fixed in formalin prior to paraffin embedding and processing for immunohistochemistry
- > Study was repeated three independent times

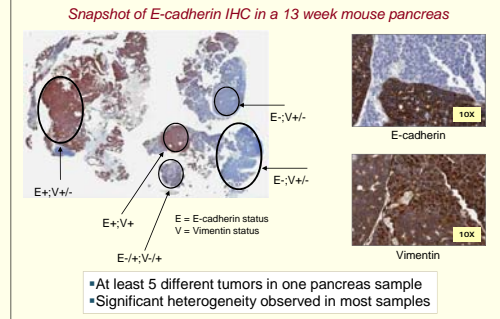
Semi-quantitation of E-cadherin and Vimentin Immunostaining:

- > An H-score was used based on subjective interpretation of the staining intensity of the chromagen-labeled antibody. Four intensity levels were used in scoring: 0 for no staining, +1 for weak/minimal staining, +2 for moderate staining, and +3 for strong staining. The relative percentage of total target cells expressing an intensity level was recorded.
- > The H-score is a sum of the percentage of cells at each scoring intensity multiplied by intensity. The range of scoring is from 0-300, from no cell staining (H-score = 0) to all cells staining at maximal (+3) intensity level (H-score = 300).

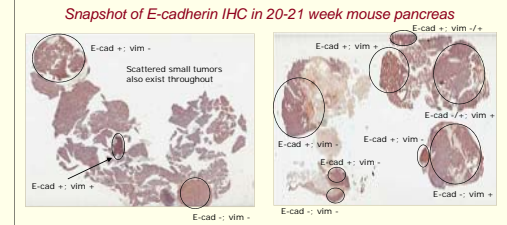
Heterogeneity of EL1-luc/EL1-SV40 T-antigen transgenic mouse tumors [1]



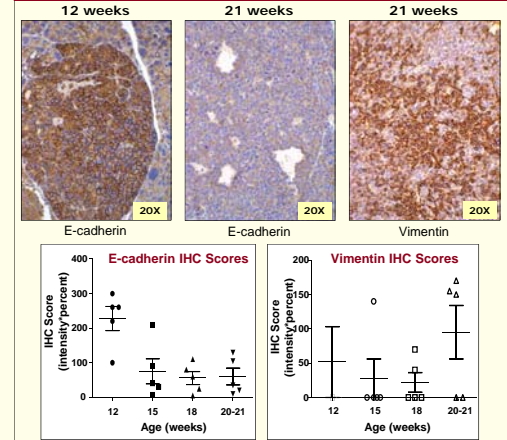
Heterogeneity of EL1-luc/EL1-SV40 T-antigen transgenic mouse tumors [2]



Heterogeneity of EL1-luc/EL1-SV40 T-antigen transgenic mouse tumors [3]



Semi-quantitation of E-cadherin and vimentin expression by IHC



CONCLUSIONS

- Tumors from EL1-luc/EL1-SV40-Tag mice expressed both E-cadherin and vimentin with a trend towards decreased E-cadherin expression over time
- Vimentin expression was highly variable but when present tended to be significantly expressed
- In many samples there was substantial heterogeneity reflected by the appearance of multiple independent tumors within each pancreas as well as significant diversity within a tumor as assessed by evaluating E-cadherin & vimentin expression ratios
- This model may recapitulate aspects of EMT *in vivo*, thus offering a system to study the signaling and molecular changes associated with EMT during cancer progression