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Purpose:

To characterize a spontaneous epithelial-to-mesenchymal conversion (EMT) in the non-transformed "functional normal" mammary epithelial cell line SCp2.

- Determine if the mesenchymal conversion in SCp2 cells is associated with tumorigenesis.
- Determine if alterations in growth factor expression and regulation are accompanied by changes in ECM and metalloproteinases expression.
- Determine if the presence of 3D structure is critical to stopping spontaneous and induced conversion.

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Purpose:

To characterize a spontaneous epithelial-to-mesenchymal conversion (EMT) in the non-transformed "functional normal" mammary epithelial cell line SCp2.

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- Determine if alterations in growth factor expression and regulation are accompanied by changes in ECM and metalloproteinases expression.
- Determine if the presence of 3D structure is critical to stopping spontaneous and induced conversion.

Technical Progress:

- Determine if the mesenchymal conversion in SCp2 cells is associated with tumorigenesis.

Rationale:

EMT-like changes have been associated with mammary gland tumorigenesis.

1. To examine tumorigenic potential, converted (SCpg2) and non-converted cells were injected subcutaneously (s.c.) into nude mice. SCp2 cells did not form tumors within 3 months after injection. SCpg2 cells were tumorigenic with a progressive increase in tumorigenicity with increasing passage number.
2. The tumors generated were further evaluated by cytohistochemistry and were found to form undifferentiated spindle cell tumors capable of invasive growth. Immunocytochemistry showed the tumors to be cytokeratin 8 and alpha smooth muscle actin negative and vimentin positive.
3. The re-cultured SCpg2 tumor cells expressed vimentin and no cytokeratins or E-cadherin.
4. Anchorage-independent growth assays showed an increase in colony formation in SCpg2 cells.
5. Due to the fact that there was increased tumorigenicity with increasing passage in culture, a cell culture tumor progression series was established. The progression series includes SCp2 cells, early passage and increasing passage SCpg2 cells.

- Determine if alterations in growth factor expression and regulation are accompanied by changes in ECM and metalloproteinase expression.

Rationale:

Growth factors and ECM molecules have been shown to induce EMT in other cell culture systems and alterations in their expression have also been implicated in malignant progression . In addition, alterations in ECM degrading proteinases have been shown to occur during tumor progression in the mammary gland.

1. There was a progressive increase in latent TGF- β in the SCpg2 cells with passage in culture as compared to the non-malignant SCp2 cells. The highly malignant late passage SCpg2 cells also expressed activated TGF- β .
2. Associated with increased tumorigenicity and TGF- β expression was altered expression of laminin-1. As determined by indirect immunofluorescence the α -chain of laminin was not expressed in SCp2 cells while both β - and γ -chains were present. While the SCpg2 cells express all 3 chains of laminin-1.
3. The expression of a complete laminin in the early transitional SCpg2 cells resulted in hormone-induced β -casein synthesis without the addition of exogenous ECM. However, continued passage in culture of SCpg2 cells resulted in the loss of hormone and ECM-induced lactogenic differentiation in the late transitional SCpg2 cells.
4. Accompanied with the above changes was the up-regulation of metalloproteinases. This included increased expression of gelatinases A and B, and two unidentified metalloproteinases (34 and 44 kd) after EMT.

- Determine if the presence of 3D structure is critical to stopping the spontaneous and TGF- β induced mesenchymal conversion

Rationale:

Loss of tissue structure and perturbed growth factor responsiveness are linked and lead to tumorigenesis

1. Exogenous addition of TGF- β and/or soluble laminin-1 resulted in increased conversion of SCp2 cells in 2-D (on plastic).
2. Pre-clustered SCp2 cells in 2-D converted when exogenous TGF- β or laminin-1 was added.
3. Pre-clustered SCp2 cells in 3-D Matrigel or collagen-1 cultures did not convert with or without the addition of TGF- β