The interaction between tumor cells and the stromal microenvironment is a critical factor in cancer development and progression. A recent study from the Khavari group profiled the expression changes during progression to invasion in a Ras-inducible model of human epithelial neoplasia and used network modeling to analyze the molecular interactions. Human dermis was seeded with H-Ras- and IκBα-expressing keratinocytes then grafted onto immune-deficient mice. The epithelial and stromal gene expression profiles were captured during progression from quiescent epithelial tissue to in situ neoplasia to invasive neoplasia. A subset of these altered genes was compiled into a “core tumor progression signature” (CTPS), which was shown to have clinical relevance in several cancer types. Network modeling of the CTPS revealed highly interconnected “hubs”, which was dominated by extracellular matrix-related genes, including β1 integrin. Targeting integrin β1 functionality reduced Ras-driven tumorigenesis in vivo and validated the network modeling strategy for predicting genes essential to neoplasia. By integrating temporal analysis of both the epithelial and stromal compartments with network modeling of molecular interactions, this work has described an effective strategy for identifying highly interconnected targets essential to tumor development.

Introduction

Recent studies have established that the stromal microenvironment influences the gene expression and behavior of epithelial tumor cells. These interactions are reciprocal, with tumor epithelium affecting stromal cells, resulting in multiple changes, including activation of cancer-associated fibroblasts, abnormalities in the tumor endothelium and tumor-associated macrophage recruitment.1-4 During tumor progression, each cellular component evolves, resulting in a complex network of changing signals, interactions and gene expression. A recent article in Cancer Cell from the Khavari group analyzed the temporal sequence of gene expression changes occurring in both the tumor and stroma during progression of normal epithelium to invasive neoplastic malignancy in a regenerated human skin model.5 Using these gene expression profiles they developed a “core tumor progression signature” (CTPS). They established the clinical relevance of this signature and used network modeling to identify highly interconnected molecular players in tumor development.

Generation of a Ras-Driven Human Invasive Neoplasia Model

To study tumor progression, the authors used a regenerated skin model produced by seeding devitalized human dermis with human keratinocytes expressing the inducible oncogene H-Ras with IκBα. The human tissue was grafted onto immune-deficient mice and allowed to heal.6 Uninduced tissue showed normal polarity and basement membrane distribution, while Ras activation induced squamous cell carcinoma-like changes (Fig. 1). These changes included increased epithelial proliferation, angiogenesis, loss of epidermal polarity, loss of basement membrane integrity and invasion of the epithelia into the underlying stroma. Laser capture microdissection was used to isolate the epithelial and stromal tissues after 0, 5, 20 and 35 days of Ras activation for microarray analysis. This analysis yielded 1,555 epithelial genes and 355 stromal genes with differential expression during Ras-induced tumor progression. The authors show distinct temporal patterns of gene changes between the epithelial and stromal compartments. The investigators further refined their gene sets by establishing that Raf was the likely downstream effector of Ras-induced tumorigenesis. Using inducible Raf/IκBα epidermis in the tumor progression model, they found that 737 differentially expressed genes were conserved between Ras- and Raf-induced tumorigenesis, thus defining a Ras/Raf “core tumor progression signature” (CTPS).

Clinical Relevance and Interactions of the CTPS

The clinical relevance of this progression signature was investigated using hierarchical data clustering of publicly available gene expression data to separate patient tumors into those “concordant”
or “discordant” with the CTPS. CTPS-concordant breast tumors showed significantly poorer survival and metastasis-free survival than the discordant tumors. Additional analyses demonstrated that the CTPS is induced during in a variety of epithelial cancers.

Based on the concept that the most highly interconnected proteins have a higher probability of being essential, the authors investigated the interactions of the CTPS genes using the Ingenuity Knowledge Base, a literature based database of molecular and biochemical interactions and associations. In this CTPS network, H-Ras and c-Jun were the most highly interconnected “gene hubs” in the induced CTPS signature. However, this network also predicted a requirement for ECM-related genes, indicating the importance of the tumor microenvironment in this model of progression.

Role of β₁ Integrin in Tumorigenesis

The third most interconnected gene in the induced gene CTPS network was the integrin β₁ subunit. Integrin β₁ protein expression was confirmed in human invasive SCC versus SCC in situ, thus validating the CTPS as an indicator of epidermal neoplastic progression. Reuter and colleagues further demonstrated that β₁ integrin was required for both initial tumor growth and continued growth of established Ras/IκBα-induced tumors, using neutralizing β₁ antibody. Both anti-β₁ treatment and shRNA-mediated integrin β₁ knockdown restored a differentiated epithelial morphology and a defined basement membrane at the tumor-stroma interface. These results establish a functional role for β₁ integrin in epithelial proliferation, differentiation and invasion during Ras-driven neoplasia. Expression analysis of genes altered during anti-β₁ treatment showed significant overlap with the CTPS, validating this approach and confirming that targeting highly interconnected “hub” genes in the CTPS could reverse neoplastic progression.

Summary and Significance

This study has provided a valuable integrated timeline of the epithelial and stromal expression changes during induction of pre-malignant hyperplasia, development of pre-invasive carcinoma in situ and progression into invasive carcinoma. The authors established a network of highly interconnected genes hubs during this process, and demonstrated that anti-tumor strategies targeting these hubs could effectively block progression.

Understanding the tumor microenvironment and the induced changes in the tumor epithelia may reveal new targets and enable more effective therapeutic strategies in the future.

Figure 1. Progression stages in human tissue neoplasia model. (A) Quiescent tissue, (B) Following Ras-IκBα induction in the epidermis in situ neoplasia develops, with loss of epidermal polarity and increased angiogenesis, followed by (C) epithelial invasion into the stroma and loss of basement membrane integrity. Figure design by Kristin E. Johnson.
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