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Medizinische Fakultät Mannheim
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Unravelling novel EMT mechanisms in colorectal cancer

Autor: Mohammed Abba
Institut / Klinik: Dept. of Experimental Surgery
Doktormutter: Prof. Dr. H. Allgayer

Tumor metastasis remains the major cause for cancer related mortality and efforts have intensified in the recent past to not only understand the molecular events driving tumor progression leading up to metastasis, but also the key players involved. This search has most often involved the use of microarrays to identify, on a genome-wide level, differentially expressed genes that distinguish cancer and normal states, and even different stages of cancer. Complicating this search, however, at least in most solid tumors including colorectal cancer, is the heterogeneous constitution of tissue which makes it almost impossible to discern, using whole tissue extracts, which cell type, and to what extent, observed deviations and expression changes can be specifically attributed to the tumor cells.

The objectives of this thesis were to:

1. Explore compartment specific molecular signatures in colorectal cancer using laser capture microdissection.
2. Corroborate the expression signatures obtained therefrom to clinico-pathologic variables in the search for novel biomarkers.
3. Identify novel deregulated genes from the differential expression analysis.
4. Investigate the functional relevance of one most significantly identified molecule as a putative regulator of colorectal cancer progression and metastasis.

In order to recapture the molecular cross-talk between tumor epithelium and stroma and the in-vivo interactions within the context of the heterogeneous tissue micro-ecology, we isolated epithelial and stromal cell populations from tumor and corresponding normal samples of patients using laser capture microdissection, and subjected these to whole genome oligonucleotide microarrays. We were able to identify and validate compartment specific gene signatures that differentiated tumor epithelium from normal epithelium, and tumor stroma from normal stroma, and to compare these expression profiles to that of whole-tissue dissection. Moreover, in this study, we present a unique perspective in which we decipher and elaborate on the key canonical pathways the different cellular compartments regulate. Accordingly, the most active pathways in the epithelial compartment were the ones that modulated cell proliferation and differentiation (p38 MAPK), gene transcription (PPAR)-alpha, tumor immune evasion (Toll-like receptor signalling), whereas the stromal pathways converged to mediate enhanced invasion, migration, angiogenesis and metastasis (CXCR4, HGF and HIF signalling).

Other results from this study include the comprehension that the tumor stroma is a significantly better index of the cancer phenotype than the cancer cells themselves, the identification of molecules with biomarker potential as supported by a comparison to a meta-analysis of colorectal cancer gene expression profiling studies, which included TGF β 1, IFITM1, CDH3 and CKB. Furthermore, we were able to identify and proffer potential molecular correlates for the CA 19-9 tumor marker that included LZTR1, TMEM201, HOXD1, LPAR1 and the DNA directed RNA polymerase II (POLR2D), all of which were significantly deregulated in the epithelial compartment. We also identified 44 genes which overlapped in all compartments, signifying a robust gene signature that cuts across cell types. A very important conclusion of the first part of this thesis is the realization that a significant amount of potentially useful information is lost when molecular profiling of heterogeneous structures fails to take into consideration the contribution of individual cell populations.

The Epithelial to Mesenchymal Transition (EMT) cascade is a complex switch driving the loss of epithelial cell polarity, and the acquisition of a motile and invasive phenotype that is functionally

associated with the formation of metastasis. An in-depth analysis of our differentially regulated gene pool of the microdissected tissues mentioned above led us to hypothesize, that an identified group of genes is involved in this process. We selected the not so well studied FOXQ1 gene that codes for a winged helical transcription factor belonging to a large family of transcription factors involved in tissue specific gene regulation for further mechanistic studies, for a novel role as a putative metastasis regulator. We were able to establish FOXQ1 as a novel mediator of colorectal cancer metastasis and were able to demonstrate that FOXQ1 is significantly up-regulated in tumor samples as compared to normal equivalents. Forced expression of FOXQ1 led to significant enhancement of tumor cell migration and invasion, while small RNA mediated silencing of endogenously high expressing cells resulted in a suppression of these processes. Moreover, using the in vivo chicken chorioallantoic membrane metastasis assay model, we showed that FOXQ1 significantly enhanced distant metastasis with insignificant effects on tumor growth. Consequently, we were able to show that it influences EMT by significantly down-regulating the expression of E-cadherin. We proceeded to identify the mechanism by which this was achieved and were able to show that FOXQ1 not only transactivates Twist1, a direct regulator of E- and N-cadherin expression, but also directly interacts with cis-elements in its proximal promoter, thereby promoting its expression. Taken together, this is the first report that shows FOXQ1 as a regulator of colorectal cancer metastasis.