Colon cancer heterogeneity: Stem cells, signals and subtypes
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Preface
Despite major advances in cancer research over the past decades, a cold fact still remains; each year cancer will affect more than fifteen million people worldwide and half of them will eventually succumb to this disease. This distressing fact has often been attributed to the heterogeneous nature of cancer that hinders our attempt for curative therapy. At least two types of heterogeneity can be considered relevant for tumorigenesis and therapy response. Firstly, individual patients presenting with cancers originating in the same organ often exhibit very dissimilar tumor behavior and a clinical course of disease. This has been dubbed *inter*-tumor heterogeneity and deals with the steady expanding assortment of cancer subtypes that can be recognized. Secondly, this contrasts to *intra*-tumor heterogeneity that mainly describes the phenotypical variation of cells within an individual cancer. Regardless of the form that is considered, heterogeneity poses a major challenge for predicting tumor behavior and clinical outcome. Therefore a concise understanding of the cellular and molecular mechanisms that are involved in tumor heterogeneity is of utmost clinical importance. This thesis attempts to generate more insights into both forms of heterogeneity for the particular case of colon cancer.

In **Chapter 1**, we present an overview of the extensive literature on both *intra-* and *inter-*tumor heterogeneity, and we will put our findings, describe in later chapters, in this context. In this chapter we present our view on the concept of *inter*-tumor heterogeneity, how this can be recognized in a malignancy and clinically exploited to optimize prognosis prediction and selection of appropriate therapeutics. In addition, we particularly focus on cancer stem cells (CSCs) as a cause of *intra*-tumor heterogeneity as the CSC model poses an attractive explanation for non-genetic diversity in tumor cell phenotype and functional features. The CSC concept has been fiercely debated over the past years. We think we have contributed to this discussion by showing that cancer cells with stem cell potential indeed exist in human colon cancer. We demonstrate in **Chapter 2** using single cell cloning experiments that at least a proportion of colon cancer cells possess both self-renewal properties and the ability to differentiate into multiple lineages. However this finding does not yet provide many insights into the biological mechanism that define cancer stemness. The latter issue is subsequently addressed in more detail in **Chapter 3** where we describe that the Wnt signaling cascade has, in analogy to normal intestinal tissue, a pivotal role in determining stem cell properties in cancer cells. Moreover, we provide evidence that the CSC phenotype is not a fixed quality but can be installed by signals emanating from the tumor microenvironment. This immediately suggests that therapies aiming to selectively target the CSC fraction are relevant but not sufficient, as more differentiated cancer cells need to be targeted as well. We summarize some key issues raised by the previous chapters on the rationale of targeting CSCs population in tumors in **Chapter 4**.

The clinical relevance of CSCs is examined in more detail in **Chapter 5** by using a set of colon cancer patients that underwent surgery in the Academic Medical Center and investigated to what extent their gene expression profiles resembled a colon CSC expression signature (generated in **Chapter 3**). Many CSC derived signatures have been previously shown to associate with poor outcome patients in several malignancies but the biological reasons underlying this association remain largely unresolved although it is often attributed to a relative high number of CSCs being present in tumor tissue of patients with a poor prognosis. We could
indeed confirm the prognostic relevance of our own CSC signatures but more importantly we provide a biological justification for their primary association with clinical outcome: an overall resemblance to a CSC signature does not simply reflect CSC numbers but points to a distinct, more immature and poorly differentiated gene expression profile of the tumor as a whole. CSC specific signatures are therefore relevant but do not provide insights per se into the true biological diversity of colon cancers. How many colon cancer subgroups can be defined and what are the molecular and clinical peculiarities associated with them? Chapter 6 deals exactly with the latter issue by interrogating and grouping colon cancers according to their gene expression profile in an unsupervised and unbiased fashion. This has resulted in the identification of three distinct molecular subtypes of colon cancer that differ extensively in both molecular and clinical properties. One of these subtypes has remained largely elusive, and its identification is of clinical interest because it tends to have a particularly poor prognosis and is refractory to existing targeted therapies. Interestingly, this poor-prognosis subtype seems to originate from a different precursor lesion compared to the majority of colon cancers. We shortly summarize our main findings in Chapter 7 and moreover speculate on the future research questions we believe are definitely in need of answers.

In conclusion, the work presented in this thesis discusses important aspects that drive tumor heterogeneity within and across colon cancers and their implications for tumor biology and clinical reality. From the formal proof of existence of CSCs in colon cancer to the unbiased identification of molecular subtypes encompassing this malignancy, we feel our work has yielded crucial novel insights into the biology of colon cancer. Nevertheless, we are only starting to appreciate the complexity of this disease and hope our results will inspire further research to better understand the molecular basis of heterogeneity and ultimately result in improved cancer treatment modalities and patient outcome.

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