Colon cancer heterogeneity: Stem cells, signals and subtypes
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ADDENDUM to CHAPTER 6

Colon Cancer Heterogeneity: From Difference to Unanimity

Manuscript in preparation
Colon Cancer Heterogeneity: From Difference to Unanimity

Colorectal cancer (CRC) is a group of heterogeneous diseases that show considerable variation in their molecular and clinical features. This heterogeneity poses significant challenges not only in patient management, but also in studying the molecular basis of the disease. We have discussed in Chapter 6 our unsupervised classification strategy to better characterize the diversities of CRC. Of note, similar independent studies [1-3] have simultaneously attempted to uncover the extent of CRC’s heterogeneity. These combined efforts highlight the need to better characterize CRCs but conversely can cause some confusion; i.e. they provide little consensus neither on the nomenclature nor on the extent of subtypes that can be identified. Here we comment on the relationship between our approach and the one proposed by Sadanandam et al [2]. We briefly describe and delineate the commonalities of our two distinct methodologies in an effort to bring more clarity into the field of CRC’s heterogeneity. In our study [4], we have employed consensus-based clustering and identified three robust colon cancer subtypes (CCS1-3) in an initial set of 90 stage AJCC II patients. This was done using a signature of 146 genes, from now on referred to as the “CCS signature”. Two main points reinforce the validity of our approach: First, we could recognize two previously well-characterized CRC subtypes; CCS1 displayed features of chromosomal instability (CIN) whereas CCS2 consisted entirely of microsatellite unstable (MSI). Secondly we have uncovered a novel subtype, dubbed CCS3, which was related to sessile serrated adenomas (SSAs) and was associated with an unfavorable prognosis and lack of response to targeted therapy. Importantly, this approach does not rely on any prior genetic or clinical information; therefore, we have an unbiased separation of patients in distinct disease entities. Using a comparable approach, the group of Douglas Hanahan performed unsupervised consensus clustering and used a non-matrix factorization (NMF) algorithm on a core set of 387 tumors to identify five distinct subtypes using a classifier of 786 genes, from now on referred to as the CRCassigner [2]. The subtypes were named according to high expression of genes associated with particular cell types in the intestine and comprise: A stem-like, Transit amplifying (TA), Enterocyte, Goblet-like and an Inflammatory subtype.

To explore the relationship between the two aforementioned CRC taxonomies, we first categorized patients from the AMC-AJCCII-90 set using the CRCassigner into five subtypes and looked at the concordance between patients classified by both taxonomies (Fig. 1a). This immediately revealed a strong correspondence of the TA with CCS1, the stem-like with CCS3, and the goblet-like and inflammatory with the CCS2 (Fig. 1b). Interestingly, the enterocyte subtype does not clearly associate with any particular CCS but rather comprises patients belonging to all three CCSs. In conclusion, both classification schemes strongly relate to each other but it is apparent that the additional subgroups defined by the CRCassigner do not merely arise from subdividing one of our CCS. To get more insight on the clinical relevance of both taxonomies we next investigated the correlation between the two classification schemes and several clinical parameters. Although the extent of association with a particular genetic hit correlated with each methodologies, CCS based classification more evidently associated with known current stratification features, such as MSI or CIMP. Importantly, this is not a consequence of subtype subdivision and subsequent loss of information but rather reflects the different strategies and signatures used between these studies (data not shown).
Finally we investigated the prognostic power of both classifiers in our AMC-AJCCII-90 dataset. As described in Chapter 6, the CCS classifier strongly relates to prognosis, but the CRCassigner only modestly associates with clinical outcome in the AMC-AJCCII-90 set. Interestingly, integration of both classification schemes revealed striking association with disease free survival especially in the CCS3. Of note, whereas CCS3-stem-like displayed a relatively good outcome, the CCS3-differentiated-like (comprising enterocytes and goblet-like) presented with very dismal prognosis (Fig. 1c). This finding is reminiscent of those described in Chapter 5 where we conclude that high expression of intestinal stem cell-related genes are actually correlated with good outcome.

In conclusion, we find that both classifications are related to each other, but it is also evident that the five subtypes defined by the CRCassigner is not a mere extension of our CCS based classification. Clinically, our subtypes are more directly related to current clinical accepted parameters (i.e MSI, CIMP, differentiation grade) but integrating both classifications provide with additional relevant insights especially in relation to clinical outcome. Many research efforts
are aimed at unraveling and characterizing the diversity of CRC but the rapid accrual of data in that area more than ever stress the need to reach a clear consensus molecular classification that would quickly reach clinical applicability and hopefully improves patient care.

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REFERENCES