Molecular understanding of KRAS- and BRAF-mutated colorectal cancers

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt försvar i Hörsal E04, byggnad 6E, Norrlands universitetssjukhus, fredagen den 5 maj, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

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Abstract

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy in both men and women, and one of the leading causes of cancer-related deaths worldwide. One frequently mutated pathway involved in oncogenesis in CRC is the RAS/RAF/MAP kinase pathway. Oncogenic activation of KRAS and BRAF occur in 30–40% and 5–15% of all CRCs, respectively, and the mutations are mutually exclusive. Even though KRAS and BRAF are known to act in the same pathway, KRAS- and BRAF-mutated CRCs have different clinical and histopathological features. For example, BRAF mutation in CRC is tightly linked to microsatellite instability (MSI) and a CpG island methylator phenotype (CIMP), which is not seen in KRAS-mutated tumours. BRAF-mutated CRCs are also more often found in right-sided tumours. However, the underlying molecular reasons for these differences have not yet been defined.

The overall aim of this thesis was to investigate molecular differences between KRAS- and BRAF-mutated CRCs to understand how KRAS and BRAF mutations differentially affect tumour progression. We used an in vitro cell culture system to explore molecular differences between KRAS- and BRAF-mutated CRCs and verified our findings using CRC tissue specimens from the Colorectal Cancer in Umeå Study (CRUMS).

We found that BRAF mutation, but not KRAS mutation, was associated with expression of the stem cell factor SOX2. Furthermore, SOX2 was found to be correlated to a poor patient prognosis, especially in BRAF-mutated cancers. We further investigated the role of BRAF in regulation of SOX2 expression and found that SOX2 is at least partly regulated by BRAF in vitro. We continued by investigating the functional role of SOX2 in CRC and found that SOX2-expressing cells shared several characteristics with cancer stem cells, and also had down-regulated expression of the intestinal epithelial marker CDX2. There was a strong correlation between loss of CDX2 expression and poor patient prognosis, and patients with SOX2 expression were found to have a particularly poor prognosis when CDX2 levels were down-regulated. In conclusion, in these studies we identified a subgroup of BRAF-mutated CRCs with a particularly poor prognosis, and having a cancer stem cell-like appearance with increased expression of SOX2 and decreased expression of CDX2.

Tumour progression is regulated by interactions with cells of the immune system. We found that BRAF-mutated CRCs were more highly infiltrated by Th1 lymphocytes than BRAF wild-type tumours, while the opposite was true for KRAS-mutated CRCs. Interestingly, we found that part of this difference is probably caused by differences in secreted chemokines and cytokines between KRAS- and BRAF-mutated CRCs, stimulating different arms of the immune response.

Altered levels of expression of miRNAs have been seen in several malignancies, including CRC. We found that BRAF- and KRAS-mutated CRCs showed miRNA signatures different from those of wild-type CRCs, but the expression of miRNAs did not distinguish KRAS-mutated tumours from BRAF-mutated tumours.

In summary, our findings have revealed possible molecular differences between KRAS- and BRAF-mutated CRCs that may explain some of the differences in their clinical and histopathological behaviour.

Keywords: colorectal cancer, BRAF, KRAS, SOX2, CDX2, cancer stem cell, Th1 lymphocytes, miRNA, prognosis