WNT signalling and the initiation of CRC

Around 70% of sporadic colorectal tumours show biallelic inactivation of the APC gene *(Adenomatous polyposis Coli)*. A high percentage of remaining tumours show activating mutations in beta-catenin or axin. These molecules are components of the Wnt signalling pathway. Activating mutations of the Wnt-signalling pathway are the only known genetic alterations present in early premalignant lesions in the intestine, such as aberrant crypt foci and small adenomas. In various animal models, activating mutations in this pathway effectively initiate tumorogenesis in the intestine in a process characterised by the formation of dysplastic crypts and adenomas similar to those found in humans. Therefore, it is widely accepted that constitutive activation of Wnt signalling caused by mutations in components of the pathway is responsible for the initiation of CRC (reviewed in Sancho et al., 2004; Figure 1).

Mutations in Wnt signalling components that lead to CRC result in the stabilization and accumulation of beta-catenin in the nucleus, and, as a result, increased transcriptional activation mediated by the beta-catenin/TCF complex. Therefore, the transactivation of beta-catenin/TCF target genes represents a primary transforming event in CRC. A few years ago we identified the genetic programme driven by beta-catenin and TCF in CRC cells. Our studies indicated that beta-catenin/TCF target genes are expressed not only in tumours but also in normal non-transformed intestinal progenitors cells at the bottom of crypts (van de Wetering et al., 2002; see Figures 2 and 3).

Our results, together with those obtained from several animal models in which Wnt signalling had been genetically manipulated (Pinto et al., 2003; Korineck et al., 1998), implied that the stem cell and progenitor compartments were controlled by Wnt signalling. These findings led us to propose that in CRC the first step towards malignancy consists of the acquisition of a crypt progenitor-like phenotype (van de Wetering et al., 2002). Our hypothesis has marked a milestone in the field and has totally changed views on the initiation of this disease. We are currently developing
animal models that will formally confirm this notion and help to shed light on why Wnt signalling mutations are an important pre-requisite for the development of CRC.

Our studies are oriented towards the identification of the nature of the founding CRC cell and the mechanisms by which it escapes cell renewal. During 2007, we have generated several DNA constructs to enable the development of animal models that will be used for this purpose. These include the conditional expression in the intestine of oncogenes involved in CRC combined with colour markers that will help to identify mutant cells. These studies may shed additional light on specific pathways that could be targeted to block the progression of this disease. Likewise, in collaboration with Eduard Batlle’s laboratory (IRB Barcelona), and by systematic analysis of colorectal samples at different stages of the disease, we aim to identify the core set of instructions imposed by Wnt signalling mutations that remains unaltered throughout the carcinogenetic process. This research will provide crucial information on the molecular targets for CRC at all stages of the malignancy.

Having identified that the initial event triggering transformation is the blockage of founder tumour cells into a progenitor phenotype, our laboratory now seeks to identify differences between the true physiological progenitors and initial founder mutant cells. To this end, during 2007, we have developed a protocol which allows us to isolate epithelial cells from the bottom of colonic crypts (ie, stem cells and early progenitors) from fresh tissue. This protocol can also be used to isolate tumour cells from early adenomas or dysplastic crypts. We are currently comparing the genetic profile of physiological progenitors with that of tumour cells from adenomas. We aim to identify tumour-specific molecular targets susceptible for targeting by the pharmaceutical industry. These targets will be particularly useful for patients suffering Familial Adenomatous Polyposis (FAP). These patients inherit a mutation in the APC gene, and due to loss of heterozygosity (LOH) they develop hundreds of polyps in the intestinal tract and are therefore predisposed to the development of malignant CRC.

**TGF-beta signalling during CRC progression**

In recent years, some of the leading scientific teams, including ours, have reported that the onset and progression of CRC can be understood using several concepts taken from the Darwinian evolution model. Under this view, the sequence of mutations acquired during CRC progression (Figure 1) can be explained by colorectal tumours evolving through a series of bottlenecks or restriction points at which only those cell cells acquiring the correct mutational event expand and progress to the next stage of malignancy. Our research interest focuses on determining how the acquisition of mutations in other signalling pathways may modulate the initial progenitor phenotype imposed by Wnt signalling to overcome the bottlenecks associated with CRC progression.

One of the most prevalent types of mutation during CRC progression are those that inactivate the TGF-beta signalling pathway (reviewed in Grady and Markowitz, 2003; Figure 1). This pathway is involved in numerous processes in the development and homeostasis of adult tissues. TGF-beta ligands activate the signalling pathway by binding to TGF-beta receptor type II homodimers. Ligand-bound receptor II recruits TGF-beta receptor I homodimers, which are
subsequently transphosphorylated and thus activated by receptor type II. Phosphorylation of the intracellular mediators smads by activated receptor I allows dimer formation with smad-4 and translocation to the nucleus, where the specific outcome of the signalling will depend on the cell type and the context of the cell itself (reviewed in Shi and Massagué, 2003). Mutations found in CRC affect mainly the TGF-beta receptor type II and the intracellular smads, smad-2 and smad-4, by abolishing the transcriptional effects mediated by TGF-beta.

Our lab currently focuses on the role of TGF-beta signalling in CRC progression. We are studying the transcriptional events controlled by TGF-beta in CRC cells. We have already identified changes in approximately 500 genes in response to TGF-beta in these cells. Unsupervised analysis of a collection of tumours of known transcriptomes on the basis of the TGF-beta signature obtained in our laboratory perfectly discriminates adenomas from carcinomas, thereby implying that these genes contain the information that drives the adenoma/carcinoma transition.

We are now dissecting this information in order to identify TGF-beta genes that play an executive role in the adenoma/carcinoma transition. Our studies in 2007 pinpoint a relevant role for TGF-beta signalling in the modification of several cellular responses required for tumour progression. Our results reveal a gain-of-function in TGF-beta signalling during CRC progression. This increase appears to translate into the expression of a series of TGF-beta target genes in several cell types within the tumour, including mesenchymal cells (see Figure 5). We have developed orthotropic models of colorectal tumours in nude mice to test the role of the TGF-beta-controlled gene signature. We are currently performing systematic shRNA-mediated down-regulation of genes contained in this signature in order to screen for TGF-beta-regulated genes that are crucial for CRC. In addition, we are studying whether the TGF-beta signature in CRC has a predictive value for clinical outcome.

**Publications**


**Other References**

Figure 4. The TGF-beta signature discriminates between adenomas and carcinomas. Unsupervised clustering analysis of a collection of tumours of known transcriptomes on the basis of target genes controlled by TGF-beta signalling clearly classifies adenomas and carcinomas in two separate branches.

Figure 5. Representative example of the expression pattern of TGF-beta target genes in colorectal adenomas (a) and carcinomas (b).


Research Networks and Grants
Molecular mechanisms involved in colorectal cancer initiation
Research Director: Elena Sancho
Start-up grant for emergent research groups
Research Directors: Eduard Batlle and Elena Sancho
Variations in the genetic program under the control of beta-catenin/Tcf during colorectal cancer progression
Fundació La Caixa, BM06-241-0: 2007-2009
Research Directors: Eduard Batlle and Elena Sancho

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TGF-beta target genes in CRC
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