Molecular mechanisms involved in colorectal cancer initiation and progression

Last year alone, more than half a million people worldwide died of Colorectal Cancer (CRC), making CRC the second cause of death by cancer. Most sporadic colorectal cancers arise from adenomas that initially are benign and occur frequently: approximately 50% of the Western population develops an adenoma by the age of 70. However, the development of a full-blown malignant colorectal tumour is a progressive process that often takes several years. During this period, the progression of the disease appears to follow a precise series of molecular events, requiring the accumulation of mutations in proto-oncogenes and tumour suppressor genes in these initially benign lesions. Access to specimens of CRC at different stages of the malignancy has allowed the analysis of the molecular alterations most frequently associated with each step of the disease (reviewed in Sancho et al, 2004). Our research aims to decipher the molecular instructions that underlie the signalling pathways that are altered in CRC and are responsible for the initiation and the progression of the disease.

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 β-catenin or axin. These molecules are all 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 tumorigenssis in the intestine in a process characterised by the formation of displastic 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). (See Figure 1.)

Mutations in Wnt signalling components that lead to CRC result in the stabilisation and accumulation of β-catenin in the nucleus, and, as a result in increased transcriptional activation mediated by the β-catenin/TCF complex. Therefore, the transactivation of β-catenin/TCF target genes is believed to represent the primary transforming event in CRC. A few years ago we undertook the identification of the genetic programme driven by β-catenin and Tcf in CRC cells. We engineered CRC cell lines bearing activating mutations of the Wnt pathway, which allowed us to block the constitutive β-catenin/Tcf mediated transcription in an inducible manner (Van de Wetering et al, 2002).

Gene expression profile analysis of CRC tumour cells before and after blockage of β-catenin/TCF activity revealed a set of approximately 100 target genes. We have analysed the expression pattern of these mole-

![Figure 1. Genetic alterations frequently associated with CRC tumour progression.](image-url)
cules in tumours and confirmed that are consistently expressed in dysplastic crypts and adenomas. Strikingly, we found that the same generic programme was expressed in normal non-transformed intestinal progenitors cells at the bottom of the crypts (Van de Watering et al., 2002). (See Figure 2.)

These observations were unexpected since at the time it was believed that de novo activation of wnt signalling in tumour cells was the initial event triggering transformation. Our additional research efforts demonstrated the presence of β-catenin, and thus physiological wnt signalling, in the nucleus of a few cells at the bottom of the crypts where the intestinal progenitors reside. 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 Watering et al., 2002). Our hypothesis has signified a milestone in the field and has completely changed views on the initiation of CRC.

Animal models in which Wnt signalling has been genetically manipulated support this notion. Mice deficient for Wnt signalling in the intestine, either through the expression of a transgene encoding the Wnt signalling inhibitor Dickkopf (DKK) or TCF4-deficient mice lack progenitors in crypts (Pinto et al., 2003; Korineck et al., 1998), whereas conditional deletion of the tumour suppressor APC leads to an abnormal expansion of the progenitor compartment of the crypts, at the expense of the compartment containing differentiated cells (Sansom et al., 2004). (See Figure 3.)

In addition, we have described that blockage of Wnt signalling in CRC cells leads to cell cycle arrest and differentiation, despite the presence of multiple other mutations in other pathways, indicating that β-catenin/TCF activity is the main mediator of CRC cell proliferation. Amongst the target genes under the control of β-catenin and TCF, we identified the oncogene c-myc as the critical molecule for proliferation of CRC cells through the repression of the cell cycle inhibitor p21[CDKN1A/CDKN1B] (Van de Watering et al., 2002). Consistent with c-myc being an essential executor of β-catenin/TCF instructions in the maintenance of the progenitor proliferative phenotype, mice deficient for c-myc in the intestine show a rapid loss of progenitors in intestinal crypts (Muncan et al., 2006).

Having identified that the initial event triggering transformation is the blockage of founder tumour cells into a progenitor phenotype, our lab now seeks to identify differences between the true physiological progenitors and initial founder mutant cells. During this year we have started a project to identi-

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**Figure 2.** Expression pattern of EphB2 (A,B) and Carbonic Anhydrase II (C,D) in early tumour lesions. EphB2, an example of a target gene under the control of β-catenin/TCF, is expressed by tumour cells from dysplastic crypts (A, black arrows), but also by normal progenitor cells at the bottom of intestinal crypts (B, black arrows). In a complementary fashion, the differentiated marker carbonic anhydrase II is expressed only in the top half of the crypts and surface epithelium (C,D black arrows), and is absent from tumour lesions (C, white arrows). This expression pattern is observed for the β-catenin/TCF target genetic programme identified so far.

- **fd** tumour-specific molecular targets susceptible to being targeted by the pharmaceutical industry. These will be particularly useful for patients suffering Familial Adenomatous Polyposis (FAP). These patients inherit a mutation in APC and as a result of a loss of heterozygosity (LOH), they develop hundreds of polyps in the intestinal tract and therefore, are predisposed to the development of malignant CRC. Our studies are also oriented towards the identification of the nature of the founding CRC cell and the mechanisms by which it escapes cell renewal. These studies may shed additional light on specific pathways that may be targeted to block CRC progression. Likewise, in collaboration with Dr. Eduard Batlle’s laboratory, we intend to identify the core set of instructions imposed by Wnt signalling mutations that remains unaltered throughout the carcinogenic process. This will be addressed through systematic analysis of CRC samples at distinct stages of the disease. The results from this analysis will yield crucial data regarding molecular targets for CRC at all stages of the malignancy.
TGF-β signalling during CRC progression

In recent years, some of the leading scientific teams, including ours, have pointed out that the emerging and progression of CRC can be explained by some concepts from the Darwinian evolution model. Under this view, the sequence of mutations acquired during CRC progression (Figure 1) can be understood as if colorectal tumours evolve through a series of bottle-necks or restriction points at which only those cell lines acquiring the correct mutational event expand and progress to the next stage of malignancy. Our research activities seek to determine how the acquisition of mutations in other signalling pathways may modulate the initial progenitor phenotype imposed by Wnt signalling in order to overcome the bottle-necks associated with CRC progression.

One of the most prevalent types of mutations found during CRC progression are those that inactivate the TGF-β signalling pathway (reviewed in Grady and Markowitz, 2003). Our lab currently focuses on the role of TGF-β signalling in CRC progression. The TGF-β pathway is involved in numerous processes in the development and homeostasis of adult tissues. TGF-β ligands activate the signalling pathway by binding to TGF-β receptor type II homodimers. Ligand-bound receptor II recruits TGF-β 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, Cell 2003). Mutations found in CRC affect mainly TGF-β receptor type II and the intracellular smads, smad-2 and smad-4, abolishing transcriptional activation or repression mediated by TGF-β.

We are currently studying the transcriptional events controlled by TGF-β in CRC cells. We have already identified changes in approximately 500-genes in response to TGF-β. Unsupervised analysis of a collection of tumours of known transcriptomes on the basis of the TGF-β signature obtained in our laboratory perfectly discriminates adenomas from carcinomas, implying that these genes may contain the information that drives the adenoma/carcinoma transition. We are now dissecting this information in order to identify TGF-β genes that play an executive role in this transition. (See Figure 4.)
**RECENT PUBLICATIONS**


**OTHER REFERENCES**


**RESEARCH NETWORKS AND GRANTS**

β-catenin/TCF target gene programs driving intestinal stem cell maintenance, colorectal cancer initiation and progression

European Union FP6 - MERG-CT-2004-006357: 2005 Principal Investigator: Elena Sancho Suils

Molecular mechanisms involved in colorectal cancer


Start-up grant for emergent research groups


Variations in the genetic program under the control of β-catenin/Tcf during colorectal cancer progression

Fundación La Caixa: 2007-2009 Principal Investigators: Eduard Batlle Gómez and Elena Sancho Suils

**COLLABORATIONS**

Giancarlo Marra (Institute of Molecular Cancer Research, Zurich, Switzerland)

Hans Clevers (Hubrecht Laboratory, Utrecht, Netherlands)
Elena Sancho’s group, March 2006.