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Wnt/FZD signaling and colorectal cancer morphogenesis

Falk Hlubek, Simone Spaderna, Otto Schmalhofer, Andreas Jung, Thomas Kirchner, and Thomas Brabletz

Department of Pathology, University of Erlangen-Nürnberg, Krankenhausstr. 8-10, 91054 Erlangen, Germany

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1. ABSTRACT

Malignant progression of colorectal carcinomas is characterized by an epithelial-mesenchymal transition (EMT)-like de-differentiation of the invading tumor cells. However a re-differentiation towards an epithelial phenotype, resembling a mesenchymal-epithelial transition (MET), is detectable in metastases. This indicates that malignant progression is based on dynamic processes, which can not be explained solely by irreversible genetic alterations, but must be additionally regulated by the tumor environment. The main oncoprotein in colorectal cancer is the Wnt-pathway effector beta-catenin, which in most cases is overexpressed due to mutations in the adenomatous polyposis coli (APC) tumor suppressor. EMT of tumor cells is associated with a nuclear accumulation of the transcriptional activator beta-catenin, which is reversed in metastases. Nuclear beta-catenin is involved in two fundamental processes in embryonic development: EMT and stem cell formation. Accumulating data demonstrate that aberrant nuclear expression of beta-catenin can also confer these two abilities to tumor cells, indicating the crucial role of aberrant Wnt-signaling for malignant tumor progression.

2. WNT-SIGNALING-BASICS

2.1. Wnt-signaling pathway

The evolutionarily conserved Wnt-signaling pathway has pivotal roles during the development of many organ systems, and its dysregulation is a key factor for the initiation of various tumors, in particular for colorectal cancer (CRC) with mutations in the APC tumor suppressor gene (1, 2). One of the fundamental results in recent colon cancer research was the demonstration, that the APC protein negatively regulates the activity of beta-catenin, (3), the main effector protein of the Wnt-pathway (summarized briefly in figure 1). Functional APC is essential for the degradation of beta-catenin, which accordingly can accumulate in APC-mutant tumor cells ((4, 5) and reviews by J. Behrens (6) and M. Bienz (7)). In epithelial cells, beta-catenin interacts with E-cadherin in adherens junctions and is involved in cell-cell adhesion, one of the main features of epithelial cells. However, after nuclear translocation beta-catenin participates in a transcriptional activator complex with members of the TCF/LEF-family and serves as the main downstream effector of the Wnt-pathway. Thus the intracellular distribution of beta-catenin is of great importance for the different functions of beta-

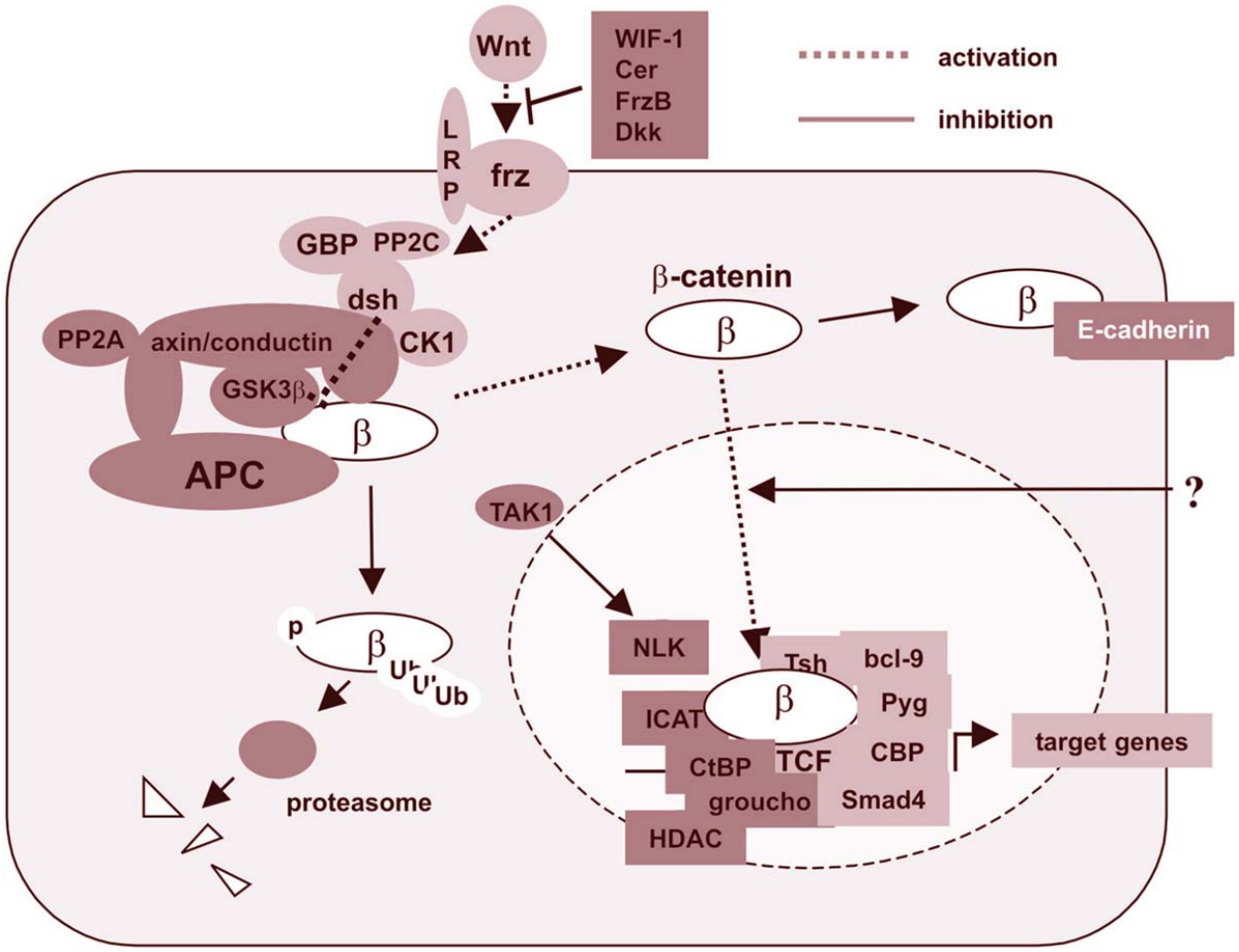

Figure 1. The Wnt signaling pathway. Wnts are secreted glycoproteins that bind to and activate frizzled (frz) seven-transmembrane-span receptors. Low-density lipoprotein receptor-related proteins LRP5/6 act as essential co-receptor. Various secreted factors, like WIF-1, Cerberus (cer), FrzB and Dickkopf (Dkk), antagonize this interaction. Wnt-signaling leads to a stabilization of cytoplasmic beta-catenin, the main effector of the Wnt-pathway. In the absence of Wnts, beta-catenin is phosphorylated (p) at the N-terminal serine and threonine residues 33, 37, 41, 45 by glycogen synthase kinase 3beta (GSK3beta), which triggers ubiquitylation and subsequent degradation in proteasomes. This is only possible in a multiprotein complex consisting of APC and the scaffolding protein axin/conductin. In the presence of Wnts, dishevelled (dsh) blocks beta-catenin degradation, possibly by recruiting the GSK3beta inhibitor GBP. Beta-catenin degradation is modulated by the casein kinase CK1 and the protein phosphatases PP2A and PP2C. Stabilized beta-catenin either is recruited to adherens junctions, or, induced by unknown signals (?), accumulates in the nucleus. This exerts its oncogenic function as a transcriptional activator after association with DNA binding proteins of the Tcf/LEF-family of transcription factors. Coactivators, such as CBP, pygopus (Pyg), bcl-9, teashirt (Tsh) support the activation of target genes (see Table 1). Phosphorylation of Tcfs by NEMO-like kinase (NLK), a target of the MAP kinase kinase kinase TAK1, as well as interaction with ICAT negatively regulate beta-catenin transcriptional activity. In the absence of beta-catenin, certain Tcfs suppress target gene transcription by interacting with the corepressors CtBP and histone deacetylase (HDAC) bound to groucho. Interaction with Smad4 might connect the Wnt- and TGFbeta-pathways. Reproduced with permission and with modification from (91).

catenin and the subsequent behavior of differentiated epithelial cells or tumor cells. TCF/LEF molecules are DNA-binding proteins, which recognize a consensus promoter sequence (TCF-binding sites: WWCAAAG), but do not have a strong intrinsic transcriptional activator function (8). In the absence of Wnt-signaling TCFs are bound by transcriptional repressors like Groucho, which keep the gene promoter inactive (9). Activation of Wnt-signaling and subsequent nuclear accumulation of beta-catenin displaces Groucho from TCF-binding. Beta-catenin/TCF plays a role in chromatin remodeling and leads to changes in promoter architecture, which makes the promoter accessible for other transcription factors. Thus, TCFs and the corresponding promoter binding sites play a key role in transcriptional regulation by integrating simultaneous signaling by various pathways (10). Although active Wnt-signaling or APC mutation in tumor cells lead to accumulation of the cytoplasmic, free pool of beta-

catenin, it is not fully understood what activates the decisive nuclear translocation of beta-catenin. A complex interaction with other pathways might be responsible.

2.2. Function in embryonic development and adult tissue homeostasis

Wnt-signaling, characterized by nuclear accumulation of beta-catenin, is involved in two fundamental processes of embryonic development and adult tissue homeostasis.

2.2.1. Stem cell formation

Wnt-signaling regulates development of various organs by controlling the function of stem cells. For example, in skin development beta-catenin is involved in hair follicle morphogenesis and control of the epidermal stem cell compartment (11-13). Of particular interest is, that the Wnt-pathway is also essential for intestinal development. Thereby, beta-catenin binds an intestine- and mammary epithelium- specific TCF-family member, termed TCF-4 (14). TCF-4 expression characterizes the intestinal stem cell compartment and targeted disruption of TCF-4 in mice leads to a severe disturbance of gut development (15). Recently, Lowry *et al.* specified the role of the Wnt-pathway in stem cell control by showing that activated beta-catenin promotes the transition from stem cell quiescence to proliferating transit amplifying progeny and further differentiation (16).

2.2.2. Epithelial-mesenchymal transition (EMT)

EMT and the reverse transition from a mesenchymal to an epithelial phenotype (MET) are fundamental processes of embryonic development and tissue morphogenesis. Wnt-signaling is involved in the induction of EMT in physiological processes like gastrulation (17) and heart cushion development (18), and when aberrantly activated, Wnt also induces EMT in tumor cells (19-22). An important hallmark of EMT is the loss of membrane-bound E-cadherin in adherens junctions. This is indicative of one of the molecular mechanism through which beta-catenin participates in EMT. Translocation of beta-catenin from adherens junctions to the nucleus may trigger loss of E-cadherin and subsequent EMT. Moreover, nuclear beta-catenin can directly transcriptionally activate EMT-associated target genes, like the E-cadherin gene repressor *slug* (23).

Compiling all data, it is becoming evident that Wnt-signaling and nuclear beta-catenin as its main effector do not simply control singular events, but regulate the complex processes of morphogenesis and organogenesis, which need temporal and spatial coordination of individual events like cell-cell attachment, migration, proliferation and differentiation, and include EMT and stem cell control. Therefore, similar to embryonic development, EMT, stem cell formation and Wnt-pathway activation (detectable by nuclear accumulation of beta-catenin) might be functionally linked in tumorigenesis.

3. COLORECTAL CANCER-BASICS

3.1. Colorectal cancers with mutated APC

Colorectal carcinomas (CRCs) belong to the most common cancers in developed countries. Most CRCs arise in a multistep process, the adenoma-carcinoma sequence, from small benign precursor lesions to metastatic carcinomas. A stepwise accumulation of genetic alterations is thought to be the driving force of this progression cascade (24). The initial and most decisive genetic alteration, which is found in more than 80% of sporadic colorectal carcinomas (25) and was initially discovered as the germline mutation in the hereditary colorectal cancer syndrome called "familiar adenomatous polyposis (FAP)", is the loss of function mutation of the APC tumor suppressor gene (1, 2), leading to aberrant activation of the Wnt-pathway (see above). The causal nature of an activated Wnt-pathway in colorectal carcinogenesis was demonstrated in different mouse models (26): The Min-mouse mutant has a truncating mutation at APC amino acid (AA) 850, and develops multiple intestinal neoplasia (Min) (27). Homozygous deletions of APC are embryonic lethal but the heterozygous phenotypes, such as targeted deletions at AA 1638, (28), AA716 (29) often develop intestinal tumors. A rapid colorectal adenoma formation is initiated by conditional targeting of the Apc gene in the intestine (truncated APC 1-580) (30). The demonstration that beta-catenin is the main effector of the canonical Wnt-pathway and that oncogenic beta-catenin mutations can be found in colorectal tumors without APC mutations, brought this molecule into the limelight of cancer research.

3.2. Colorectal cancers with normal APC

Like the FAP type of hereditary colorectal cancer, most sporadic colorectal carcinomas arise from adenomas and have APC mutations as initial genetic alterations. The second major group are the replication error (RER) positive carcinomas, associated with microsatellite instability (MSI). The "Hereditary Non-Polyposis Colorectal Cancer (HNPCC)"-syndrome and about 10% of sporadic colorectal carcinomas fall into this group, which is also characterized by a different morphology and clinical prognosis. About half of these carcinomas have normal APC genes (31), however the general importance of a dysregulated Wnt-pathway and its main effector beta-catenin in colorectal cancer formation is indicated by the fact that mutations in other components of this pathway, leading to enhanced beta-catenin activity, are found in a high percentage of such tumors. In particular, dominant mutations at the target serine and threonine residues for GSK3-beta in the beta-catenin gene itself are found in up to 27 % of microsatellite instable carcinomas, leading to a stabilisation of the molecule (32). *Loss of function* mutations in the *conductin/axin-2* gene were demonstrated in 25% of MSI carcinomas, and like APC mutations showed reduced beta-catenin degradation (33). Moreover inactivating frameshift mutations in the Tcf-4 gene were found in 39% of human microsatellite instable colon carcinomas (34) how are these going to activate CRC?. The important role of the dysregulated Wnt-pathway in colon carcinogenesis is also indicated by the fact that ulcerative colitis associated colon carcinomas show APC gene mutations and nuclear accumulation of beta-catenin (35). Thus, a dysregulation of the Wnt-pathway by targeting APC, beta-catenin or other components is found in almost all colorectal carcinomas.

4. WNT-SIGNALING AND COLORECTAL CANCER

This review is focused on the role of Wnt-pathway activation in malignant tumor progression (invasion and

metastasis). Its main role is in embryonic development and maintaining adult homeostasis of adult intestinal tissue, while aberrant activation of the Wnt-pathway - leads to formation of benign adenomas and is the initial and decisive trigger of intestinal tumorigenesis. This part is summarized in the following chapter, for more extensive coverage of Wnt-signaling in early colorectal carcinogenesis please refer to the following reviews (36-38).

4.1. Wnt-signaling in early colorectal carcinogenesis

Mainly based on the work by the group of Hans Clevers, it is now clear that regulated spatial activation of the Wnt-cascade is crucial for the correct architecture of both small and large intestinal mucosa (39-41). Thereby Wnt-signaling is restricted to the crypts of the small intestine and to the crypt base of the large intestine and controls strict compartmentalization along the crypt-villus axis into stem cells, proliferating transit amplifying units and differentiated cells (resorptive, goblet, enteroendocrine cells) (38). In particular as shown in skin epithelium, Wnt triggers the transition from stem cells to proliferative progenitor cells in transit amplifying units (16). With this basic function in mind, it becomes evident that aberrant Wnt-pathway activation, no more restricted to its physiological niches, is responsible for both uncontrolled proliferation and disturbed tissue architecture, and initiates the formation of colorectal adenomas. Physiological Wnt-target genes (Table1) that are activated in proliferating transit amplifying units include c-myc (42) and cyclin D1 (43, 44). Wnt-target genes, regulating epithelial cell migration and compartmentalization along the crypt-axis are EphB sorting receptors (39, 40). Also *survivin*, which is considered as a stem cell marker and is also overexpressed in CRC, was defined as a direct beta-catenin target (45). These data support the role of aberrant Wnt-pathway activation in the formation of tumor stem cells. Aberrant nuclear accumulation of beta-catenin can be already found in earliest benign lesions (although at low levels) and subsequent activation of these target genes can explain both hyperproliferation and disturbed mucosal architecture detectable in such lesions.

Upregulation of the TCF/beta-catenin driven crypt progenitor program seems to be necessary to induce proliferating tumors throughout colorectal carcinogenesis from early adenomas to metastasizing carcinomas, but alone is not sufficient to allow malignant tumor progression (46). This is achieved by additional loss of the epithelial phenotype of tumor cells in carcinomas, which is still preserved in proliferating adenomas and parts of the carcinomas as indicated by formation of adherens junctions and tubular differentiated structures. But even in this process aberrant Wnt-pathway activation might be involved by inducing EMT, its second important function in embryonic development.

4.2. Wnt-signaling in the malignant progression of colorectal cance

4.2.1. Intratumorous heterogeneity in phenotype and beta-catenin expression

Given the two important, contrary roles of beta-catenin in either the E-cadherin dependent determination of the epithelial phenotype (membranous localization) or as transcriptional regulator and main effector of the Wnt-pathway (nuclear localization), its function as an oncoprotein in APC-mutant colorectal cancers takes shape. Indeed nuclear beta-catenin is detectable in human FAP-associated and sporadic colon adenomas and adenocarcinomas (47). However, the amount of nuclear beta-catenin is increasing from early adenomas to adenocarcinomas (48). Moreover, a hallmark of typical CRCs is their intratumorous heterogeneity (?), both at morphological and molecular level: central tumor areas are often more differentiated, showing tubular structures built up by polarized tumor cells. In contrast tumor cells at the invasive front of the tumor-host interface often seem to have undergone EMT and are de-differentiated. These phenotypical changes are reflected by the heterogenous distribution of beta-catenin within an individual tumor (figure 2A): In most well- to moderately differentiated colon adenocarcinomas nuclear beta-catenin is predominantly accumulated in de-differentiated tumor cells at the invasive front, which have undergone EMT. In contrast in central differentiated areas beta-catenin is often located at the membrane and nuclear accumulation is hardly detectable (49, 50). In cell culture experiments it was shown that beta-catenin activation can directly induce loss of epithelial differentiation and EMT (21, 22). This fact further indicates a functional connection of strong nuclear beta-catenin accumulation and EMT in budding tumor cells at the tumor host-interface.

An increasing number of identified beta-catenin target genes, whose expression is strongly enhanced in these tumor areas and which are known to code for regulators of differentiation and effectors supporting invasion and dissemination, supports a direct connection (Table 1): A recent publication clearly demonstrates a direct link between avtivated beta-catenin and EMT. Conacci-Sorell *et al.* described *slug*, a strong inducer of EMT in embryonic development, as a target gene of beta-catenin (23). Moreover, *Cdx-1*, encoding a homeobox-factor (51), *Id2* (Inhibitor of differentiation-2) (52) and *ENC1* (53) were identified as beta-catenin/TCF target genes. All three proteins inhibit epithelial differentiation and keep cells in a less differentiated, stem cell-like state. An activation of such genes by nuclear beta-catenin could explain the de-differentiated phenotype of nuclear beta-catenin expressing colon cancer cells at the invasive front. Other genes regulated by nuclear beta-catenin code for direct effectors of colon cancer progression, like *urokinase-receptor* (*uPAR*) (54), MMP-7/*matrilysin* (55, 56)*, c-jun* (54)*, ets2 (57), tenascin-C* (58), *fibronectin* (59)*, laminin-5 gamma2 chain* (60)*, MT1-MMP (61), CD44* (62), VEGF (63) and FGF-2 (unpublished results). Both uPAR and matrilysin are overexpressed by the tumor cells and facilitate extracellular matrix proteolysis, which allows detachment and motility enhancement of the tumor cells. The isolated gamma2 chain of laminin-5 is overexpressed selectively in dedifferentiated carcinoma cells at the invasive front (figure 3) (64) and after cleavage by MT1-MMP fragments are potent inducers of epithelial cell migration, e.g. in wound healing and embryonic

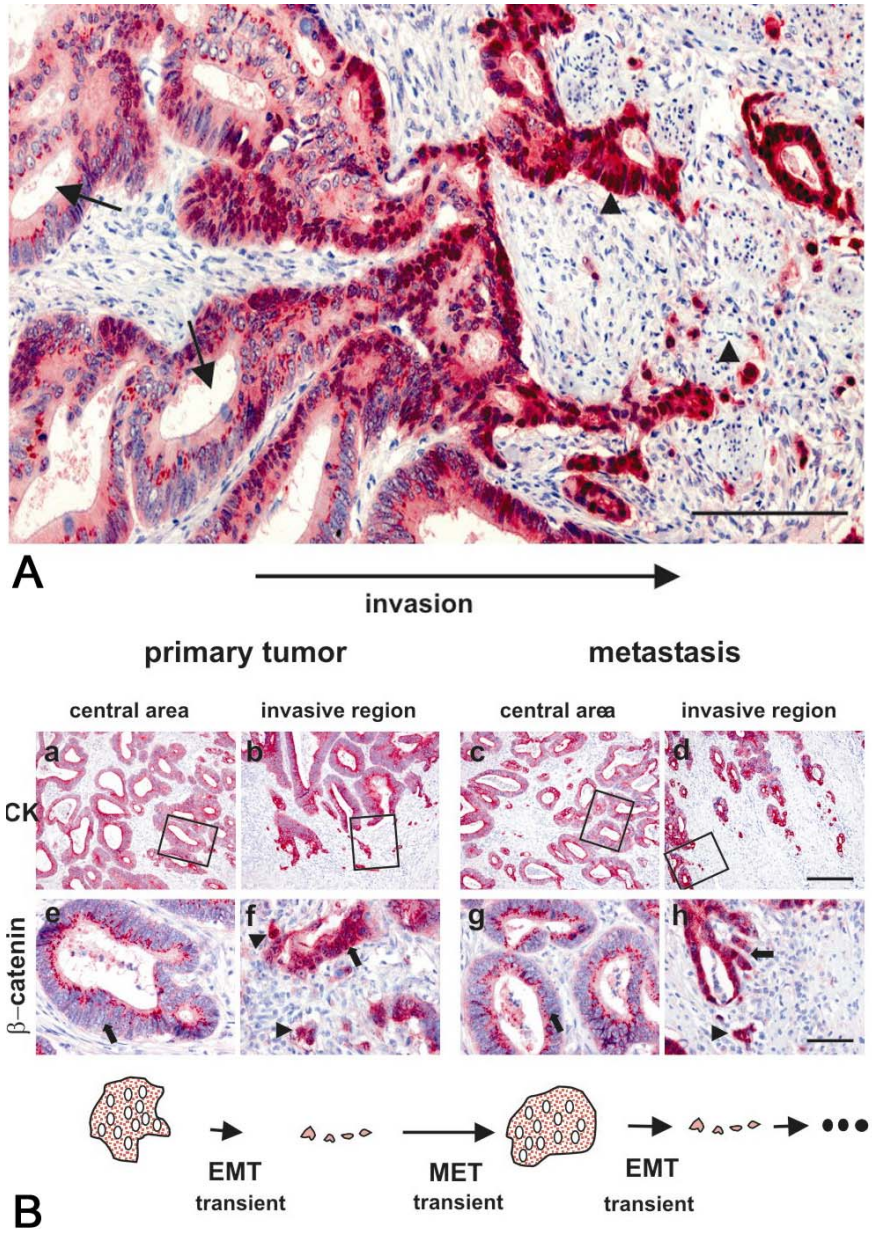

Figure 2. EMT and nuclear accumulation of beta-catenin at the tumor host interface and reversal in metastases. (A) Immunohistochemical staining of a colorectal carcinoma for beta-catenin (red, nuclear counterstaining in blue). Towards central areas (left) the tumor exhibits differentiated qualities, characterized by tubular structures built up of polarized epithelial tumor cells lacking nuclear beta-catenin (arrows). In invasive regions tumor cells change their morphology and undergo EMT: dissociation towards single, disseminating tumor cells with strong accumulation of nuclear beta-catenin (arrowheads). (B) Reversal in metastases: Shown are central areas (first column) and invasive front (second column) of the primary tumor and central areas (third column) and invasive front (fourth column) of the corresponding metastasis. Stainings are for CK-18 (first row), beta-catenin (second row). Boxes indicate magnified regions in stained serial sections. Specific staining is red, nuclear counter-staining is blue, size bar is 200 µm (a – d) and 40 µm (e – h). CK 18 stainings show a differentiated growth pattern with tubular structures in the centers of primary tumor (a) and metastasis (c) and loss of tubular growth and tumor cell dissemination in the corresponding invasive fronts (b and d). Tumor cells are clearly polarized in the differentiated central areas in both primary tumor and metastasis, and lack nuclear beta-catenin (arrows) (e and g). In contrast tubules at the invasive front break up, tumor cells loose their polar orientation (arrows) and are dissociated (arrowheads) (f and h). This is accompanied by a nuclear accumulation of beta-catenin. A scheme depicting the deduced model of tumor progression is shown below: ongoing dynamic changes in tumor cell phenotype by transient EMT/MET processes allowing permanent switches of tumor growth and dissemination. A driving force could be regulatory input from the changing tumor environment.

Table 1. Beta-catenin target genes relevant to cancer

TARGET GENE	FUNCTION
slug	EMT-inducer
MMP-7, MMP-26, MT1-MMP, UPA-R	protein degradation
laminin gamma2 chain, fibronectin, tenascin C	ectracellular matrix
CD44	dissemination
Cdx1, Id2, Enc-1	loss of differentiation
c-myc, yclin D1, securin	proliferation
c-jun, ets2, fra-1, ITF-2	oncogenic transcription factors
VEGF	angiogenesis
BMP-4, ephrinB2/B3	morphogenesis
Gastrin, PPARdelta	trophic factors
MDR, survivin	cell survival. stem cell formation
conductin/axin-2, Tcf-1	negative feedback and tumor suppression

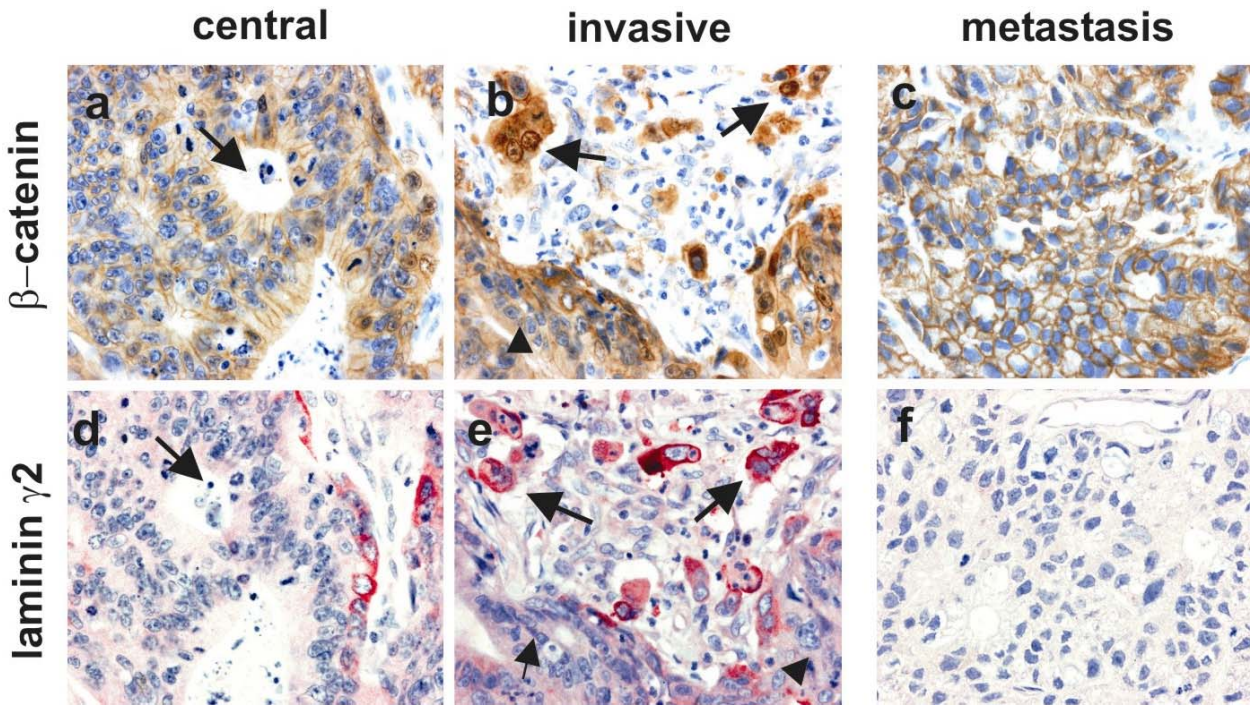

Figure 3. Correlated expression of beta-catenin and invasion-related target genes in colorectal cancer progression. Shown is the immunohistochemical staining of serial sections for beta-catenin (a-c, specific staining in brown, nuclear counterstaining in blue) and one of its invasion-associated targets genes, laminin gamma2 chain (d-e, specific staining in red) in a colorectal adenocarcinoma (a and b, d and e) and a corresponding liver metastasis (c, f). In central tumor areas (a, d) an epithelial differentiation, characterized by polarized tumor cells forming tubular structures (arrow), is retained. These tumor cells, like normal colon epithelial cells, express membranous beta-catenin and do not overexpress the laminin gamma2 chain. In invasive regions, dedifferentiated tumor cells detach from the primary tumor into the surrounding tissue, loose membranous but accumulate nuclear beta-catenin (arrows) and overexpress the laminin gamma2 chain. Tumor cells towards the tumor center still lack nuclear bata-catenin (arrowhead) and its target gene. In growing metastases tumor cells regain their epithelial phenotype, reexpress membranous beta-catenin but lack nuclear beta-catenin (c) and laminin gamma2 chain (f).

development (65). Fibronectin is considered as a mesenchymal marker and can be detected in the budding tumor cells (66). Recently we described that L1, an axon guiding factor, is activated by beta-catenin and aberrantly expressed in tumor cells at the invasive front (67). The oncoprotein c-Jun, a component of the transcription factor AP-1, is itself another strong transcriptional activator of invasion factors like uPAR, matrilysin and laminin-5 gamma2. A similar role is described for ETS-transcription factors (68).

Another important process in tumor growth and invasion is the modification of surrounding tumor stroma. The stroma and the stromal cells participate directly in tumor growth and invasion by producing various degrading enzymes like MMPs, by storing cytokines and also supply the tumor with blood vessels (69). Both VEGF and FGF-2, activated by nuclear beta-catenin, are cytokines involved in the generation of the tumor stroma and tumor angiogenesis (70). Splice variants of CD44 (e.g. v6) are known

Table 2. Environmental factors regulating directly or indirectly the intracellular distribution of beta-catenin and activating EMT

FACTORS	MOLECULAR EFFECTS	CONSEQUENCES
cytokines (TGFbeta, TNFalpha, TFF, IGF, EGF, HGF) extracellular matrix MMPs	tyrosine phosphorylation of beta-catenin activation of ILK activation of EMT-inducers (snail, slug,...) cleavage of e.g. E-cadherin	nuclear accumulation of beta-catenin induction of EMT nuclear accumulation of beta-catenin inhibition of E-cadherin transcription induction of EMT loss of E-cadherin function

to directly support dissemination of isolated tumor cells and are associated with the presence of metastases and an unfavorable prognosis of colorectal cancer (71). Finally, a translocation of membraneous beta-catenin to the nucleus leads to a loss of E-cadherin function. This allows further detachment of tumor cells from epithelial cell complexes and supports the loss of epithelial features. Thus, accumulation of nuclear beta-catenin in invading tumor cells is directly involved in associated EMT.

4.2.2. Re-differentiation of tumor cells in metastases

Strikingly, the whole process of nuclear accumulation of beta-catenin and associated EMT is reversed in metastases, since strong expression of nuclear beta-catenin is not found in the central areas of many metastases, but tumor cells recapitulate the differentiated epithelial phenotype of the primary tumor (figure 2B) (72). This is associated with downregulation of invasion-related Wnt-target genes, e.g. laminin-5 gamma2, in central differentiated areas of metastases (figure 3). However, at the invasive rim of metastases nuclear beta-catenin and EMT is detectable again (figure 2B). An apparent drawback is that EMT is reversed during metastasis formation, indicating ongoing switches between EMT and mesenchymal to epithelial transitions (MET) during tumor progression. Why should de-differentiated, disseminating tumor cells undergo a MET? A reduction of proliferative activity in dissociating tumor cells expressing high amounts of nuclear beta-catenin was found (72). Although these tumor cells overexpress the beta-catenin target gene *cyclin D1*, which is associated with proliferation, they also show a parallel overexpression of the cell cycle inhibitor $p16^{INK4a}$ was described, which could explain the observed growth arrest (73, 74). Overexpression of p16 seems to be initiated by nuclear beta-catenin in tumor cells at the invasive front (unpublished results). Obviously, in well-differentiated CRCs a loss of epithelial capabilities is coupled with a shut-down of proliferation. Accordingly, in order to expand metastatic growth, disseminated de-differentiated tumor cells of well differentiated carcinomas must regain their epithelial function. Recently it was shown that Wnt-signaling itself may be necessary to induce MET, since knockdown of the Wnt-receptor FZD7 inhibited epithelial re-differentiation of colorectal cancer cells after transient EMT induction (Vincan *et al.*, submitted).

Taken together nuclear beta-catenin expression in CRCs is characterized by both a temporal increase during colorectal carcinogenesis (from early adenomas to carcinomas) and a spatial heterogeneity within an individual tumor (reversible, maximum in invasive, EMT-associated tumor cells).

4.2.3. Environmental regulators of intracellular beta-catenin localisation and EMT induction

Since all tumor cells in an individual tumor harbour APC-mutations, a nuclear accumulation of beta-catenin can not be due to this alteration alone, but its intracellular distribution within different tumor areas has to be explained by additional events. Due to the observed dynamic changes in intracellular distribution of beta-catenin and the tumor cell phenotype, a main driving force of these processes must be the tumor environment acting on the genetically altered tumor cells.

Cell culture experiments revealed a role of environmental factors, including cytokines and extracellular matrix, on the intracellular beta-catenin distribution and function, as well as on induction of EMT (Table 2). Intestinal trefoil factor (TFF3) (75), insulin-like growth factors (IGF I and IGF II) (76), epidermal growth factor (EGF) (77) and hepatocyte growth factor/scatter factor (HGF) (77) lead to a tyrosine phosphorylation of beta-catenin with subsequent perturbation of E-cadherin binding, loss of intercellular adhesion and promotion of cell motility. By affecting the function of beta-catenin, an overexpression of these cytokines in colon carcinomas is thought to modulate tumor cell adhesion and migration. Overexpression of IGF II enhanced colon tumor growth in a mouse model (78) and induced a nuclear translocation of beta-catenin coupled with an EMT in bladder and mammary carcinoma cell lines (79). Moreover a combination of TGFbeta- and TNFalpha-treatment lead to a direct EMT in colorectal cancer cells (80).

Epithelial-mesenchymal interactions are decisive for intestinal development (81). Thus, mesenchymal factors, in particular components of the surrounding extracellular matrix, could have a potent regulatory effect on tumor cells, which might have a reactive competence similar to embryonic epithelial cells due to APC mutations affecting the WNT-pathway. In this context the perhaps most significant regulator of intracellular beta-catenin distribution is the integrin linked kinase (ILK), a serine/threonine kinase which binds to beta1- and beta3-integrins. After binding of extracellular matrix proteins to their integrin receptors, ILK is activated and exerts various intracellular effects. One is the induction of a nuclear translocation of beta-catenin and subsequent activation of the beta-catenin/TCF transcriptional activator (82). Moreover it was shown that ILK stimulates the

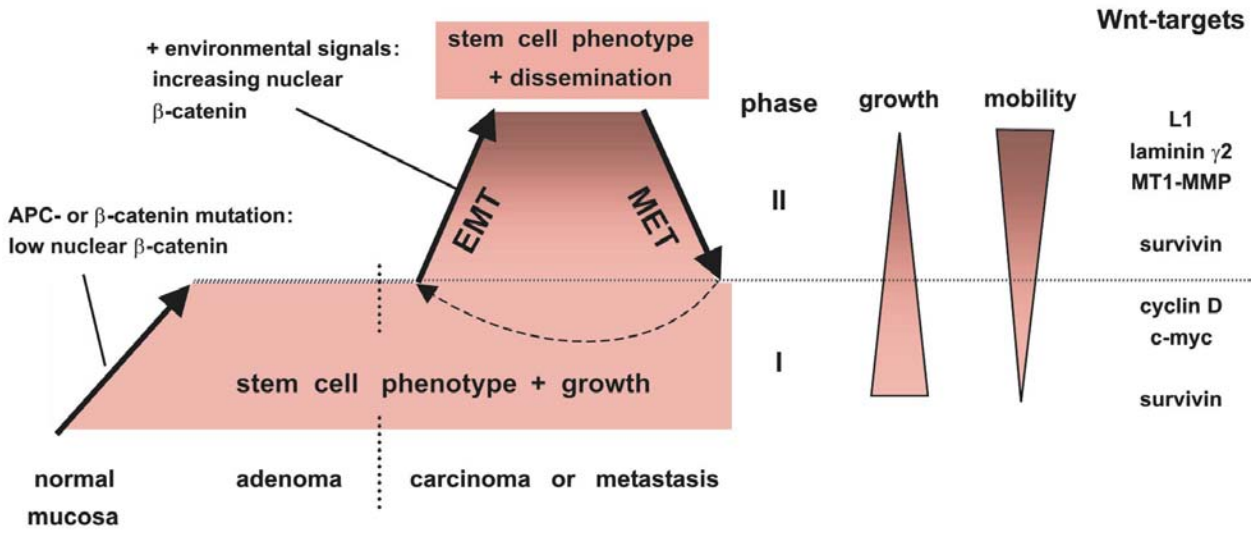

Figure 4. Scheme of dynamic tumor progression. Data deduced from expression analyses of human tumors are summarized to a two-phase model: Inappropriate activation of stemness in selected tumor cells and subsequent proliferation and epithelial differentiation in the main tumor mass can be detected in all steps from early adenomas to metastases (phase I allowing tumor growth). So, low amounts of beta-catenin might be sufficient for a persistent activation of stemness in all steps of tumor progression. Tumor cells are attached to each other in an epithelial context. Transient activation of phase II (possibly by aberrant environmental signals) in both primary carcinomas and metastases is the hallmark of malignancy and gives tumor cells (including cancer stem cells) a transient mobility, favoring dissemination. Its cycling dynamics is indicated by transient expression of epithelial to mesenchymal transition (EMT)-associated genes, which can be reversed by a mesenchymal to epithelial transition (MET) leading to epithelial redifferentiation. A potential restart of EMT is indicated. Of note, stem cell markers can be detected in both phases. Reproduced with permission and with modification from (90).

transcriptional repressor and EMT-inducer *snail* (83). Thus ILK-activation may be directly involved in the observed EMT of nuclear beta-catenin expressing tumor cells at the invasive front. Moreover, it was shown that contact of colorectal cancer cells with collagen type I can induce a transient loss of intestinal differentiation though a beta1-integrin/FAK transmitted downregulation of the intestine specific homeobox factor Cdx2 (84). Also other pathways known to be altered in colorectal cancers, like the PTEN/Akt (85)- or the TGFbeta-pathway (86), are thought to interfere with the Wnt-pathway and possibly the nuclear accumulation of beta-catenin. However, the relevance of these interactions for colorectal carcinogenesis is still unclear.

Also indirect effects by modulating beta-catenin associated proteins could be relevant for the intratumorous heterogeneity of beta-catenin distribution. For instance, colon carcinomas show an ectopic nuclear overexpression of LEF1, which belongs to the TCF/LEF-family of DNA-binding proteins. Like TCF-4, LEF1 binds beta-catenin, and it is discussed that increasing nuclear LEF1 can trap beta-catenin in the nucleus (87). Also, E-cadherin may be the direct target for environmental factors like MMP-7, which cleaves membranous E-cadherin (88), and of ILK activators, leading to a repression of E-cadherin transcription (83). E-cadherin mutations are not common in colorectal cancers (89), but the decreased E-cadherin expression observed at the invasive front may be due to such environmental factors. Indirectly, this could increase the cytoplasmic free pool of beta-catenin in addition to APC-mutations, which subsequently can be translocated and trapped in the nucleus of the carcinoma cells.

5. CONCLUSIONS AND PERSPECTIVES

Almost all CRCs show aberrant activation of Wnt-pathway, mainly due to APC-mutations. This might have two decisive effects in colorectal carcinogenesis (summarized in a two phase model of tumor progression in Figure 4):

1. Aberrant Wnt-signaling initiates uncontrolled proliferation and disturbance of tissue architecture: Increasing, uncontrolled Wnt-signaling induces persistence and distribution of intestinal stem cells and subsequent activation of the crypt progenitor program in all stages of colorectal carcinogenesis.

2. Aberrant Wnt-signaling promotes malignant progression: Stronger accumulation of nuclear beta-catenin, eventually triggered by tumor environmental signals, leads to a transient loss of epithelial differentiation and EMT at the tumor stroma interface and favors tumor cell dissemination.

Since the loss of differentiation/EMT at the tumor stroma interface is reversed in most metastases, an important role of tumor environment in tumor progression is indicated. Furthermore it suggests, that malignant progression of CRC is a regulated, dynamic process involving transient stages of EMT and MET. At the

molecular level the changes in tumor cell phenotype are associated with dynamic changes in the intracellular distribution of beta-catenin, the main oncoprotein in colorectal carcinogenesis. Nuclear beta-catenin is involved in two fundamental processes in embryonic development: EMT and stem cell formation. Based on accumulating data we suggest that aberrant nuclear expression of beta-catenin can confer these two abilities also to tumor cells. The unusual combination of EMT and stem cell competence might result in a pool of "migrating tumor stem cells", which drive tumor invasion and metastasis (90).

6. ACKNOWLEDGEMENTS

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Abbreviations: CRC (colorectal carcinoma), EMT (epithelial to mesenchymal transition), MET (mesenchymal

to epithelial transition), FAP (familiar adenomatous polyposis), APC (adenomatous polyposis coli),

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Send correspondence to : Thomas Brabletz, Department of Pathology, University of Erlangen-Nürnberg, Krankenhausstr. 8-10, 91054 Erlangen, Germany, Tel: 49/9131 8522856, Fax: 49/9131 8524745, E-mail: thomas.brabletz@patho.imed.uni-erlangen.de

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