7

Molecular Traits of the Budding Colorectal Cancer Cells

Boye Schnack Nielsen
Bioneer A/S, Kogle Allé, Hørsholm, Denmark

1. Introduction

The process of cancer cell metastasis is one of the latest steps in cancer progression that involves escape from the primary tumor through the vascular system to local lymph nodes and distant organs, recently reviewed by Chaffer and Weinberg (Chaffer & Weinberg, 2011). For a cancer cell to metastasize, it must escape the primary tumor by obtaining features that allows detachment from neoplastic epithelial structure, invasion through extracellular matrices, intravasation, survival in the blood circulation, extravasation, establishment or *homing* in a novel organ and local *de novo* proliferation. Colorectal cancers (CRC) comprise different subtypes and vary in the degree of differentiation as well as in the local invasion pattern in the tumor periphery. The invasion pattern is partly related to the metastatic ability of the tumor, and the invasion pattern can be fully discerned by microscopy analysis. Molecular characteristics related to the invasion pattern may help in the histopathological diagnosis to provide a prognostic perspective for the patient and potentially identify which patient would benefit from a certain therapy. For CRC, an invasion pattern related to the ability of cancer cells to form buds and focal single cell invasion into the neighboring stroma has obtained much attention. The invasive cancer cells are known as budding cancer cells and the budding phenomenon describes a morphological event, which is becoming better characterized at the molecular level. In this chapter, I will go through some of the molecular characteristics linked to the budding cancer cells and link the observations to the morphologic and molecular changes related to epithelial-to-mesenchymal transition (EMT) often assigned to locally disseminating cancer cells.

1.1 Identification of budding colorectal cancer cells

Two types of metastatic processes can be considered: active and passive. Passive metastasis occurs when cancer cells enter the vascular system, for example by being captured by disrupted vessels in the tumors, and subsequently being trapped in microvessels for example in the liver or lung, where the cancer cells initially proliferate within the vessel and later on disseminate into the parenchyma of the organ. Active metastasis is considered to involve a certain level of EMT in the primary tumor followed by invasion into the vascular system, extravasation by crossing the vascular wall and invasion into the new organ, and then reverting to an epithelial cancer cell capable of *de novo* proliferation and differentiation to form new tumors.

Budding cancer cells likely belong to the category of active metastasis process because their prevalence is directly linked to metastasis and is independent of the TNM classification (Nakamura et al, 2005; Okuyama et al, 2003; Prall et al, 2005; Ueno et al, 2002). Japanese histopathologists have many years tradition for evaluating growth and invasion patterns in CRC including addressing the clinical implications (Fujimori et al, 2009). A clinical significance of budding cancer cells was first described in colon cancers in 1993 by Hase *et al* (Hase et al, 1993). Hase et al (Hase et al, 1993) defined the budding cancer cells as "small clusters of undifferentiated cancer cells located ahead of the invasive front of the lesion".

Hase et al (Hase et al, 1993) evaluated the degree of budding cancer cells in normal hematoxylin and eosin (H&E) stained sections, an approach being widely used by others (Nakamura et al, 2005; Okuyama et al, 2003; Ueno et al, 2002). Because the budding cancer cells are often present in a dense desmoplastic or highly inflamed stroma and because budding cancer cells can acquire morphologically odd shapes (see below), a precise identification of budding cancer cells in H&E stained sections may not always be straight forward (Turner et al, 2007) at least for non-pathologists. Later studies have employed cytokeratin immunohistochemistry whereby budding cancer cells are much easier identified (Prall, 2007; Prall et al, 2005; Turner et al, 2007; Zlobec et al, 2010). The use of cytokeratin immunohistochemistry to detect cancer cell budding versus evaluation in H&E stained sections may lower the proportion of misclassified cases.

The prevalence of tumor cell budding varies strongly from tumor to tumor and Hase et al (Hase et al, 1993) stratified tumors into BD-1 (none or mild) and BD-2 (moderate or severe) based on the H&E stained sections. Prall et al (Prall et al, 2005) stratified tumors into low and high budding based on sections immunohistochemically stained for cytokeratins and counted all cytokeratin-positive cancer cell clusters with less than 5 nuclei. Hase et al (Hase et al, 1993) classified approximately 25% of all CRC analyzed as BD-2 and Prall et al (Prall et al, 2005) classified approximately 30% as high level budding.

Cancer cell budding as it is observed in colon and rectal adenocarcinomas should not be confused with the diffuse growth pattern, which is common in other gastrointestinal cancers and in contrast to tumors with a solid (or expanding) growth pattern. CRC showing a diffuse growth pattern are low differentiated neoplasms and do not show signs of tubular or glandular formations. Therefore quantitative estimation of cancer cell budding even done using cytokeratin immunohistochemistry should be done with some caution.

The degree of cancer cell budding as stratified into groups with none, mild, moderate and strong budding, or less or more than 5 cells in a cluster, indicate that cancer cell budding is not an "all or none" phenomenon, but reflects gradual differences. Nonetheless, both studies (Hase et al, 1993; Prall et al, 2005) showed that tumors with the highest level of budding significantly more often linked to lymph node metastasis than tumors with a low level of budding. The highest level of budding was, not surprisingly, seen in patients with the poorest survival rate, however, cancer biology reflects individual heterogeneity and in fact, some tumors with low budding also show metastatic events (Hase et al, 1993; Prall et al, 2005; Tanaka et al, 2003; Ueno et al, 2002).

1.2 Histo-morphological characterization

One of the morphological features of the budding cancer cells in CRC is their characteristics of dedifferentiation and acquisition of odd shapes as described at the ultra-structural level by Gabbert et al in 1985 (Gabbert et al, 1985). These authors studied DMH-induced colon

cancers in rats and found that poorly differentiated tumors, which showed frequency of lymph vessel invasion, also had isolated cancer cells along the invasive front. Gabbert et al (Gabbert et al, 1985) states about the cancer cells located ahead of the invasive front that "Their nuclei are very large and at their cell surface show no signs of differentiation such as formation of microvilli or formation of a basement membrane are discernible." Thus the cellular characteristics of isolated cancer cells suggested general dedifferentiation compared to the cancer cells placed within the adjacent glandular structures. In addition, the isolated cancer cells at the invasive front showed overt cell shape: "The cell shape of more or less isolated tumor cells at the foremost invasion front is extremely variable … ranging from a round or oval to a sand glass-like." These observations are consistent with the budding cancer cells in human CRC identified both in H&E stained sections as well as cytokeratin immuno-peroxidase stained sections, see for example the studies by Prall and Turner et al (Prall, 2007; Prall et al, 2005; Turner et al, 2007). According to the histological characteristitcs of the budding CRC cells at the invasive front, the stepwise process of budding-directed growth can be divided into the following steps 1) the budding cancer cells detach from the glandular structures, 2) morphologically change cell shape and 3) invade a short distance, say up to 400μm, through the adjacent tumor-associated stroma and 4) settle and 5) found novel glandular structures. Budding CRC cells are rarely seen in the central areas of the tumors, suggesting that the local environment, the stroma constituting the tumor periphery, is dictating the budding process together with the cancer cells. Interestingly, the morphological characteristics of dedifferentiation and dynamic change in cell shape are consistent with characteristics of cells undergoing EMT as described for cultured cells (Kirkland, 2009; Thuault et al, 2006).

Together with the findings that the prevalence of budding cancer cells is linked to more metastatic cancers, strongly suggests that the budding cancer cells also possess cancer stem cell activity. Thus the budding CRC cells possess all canonical requirements for actively metastasizing cancer cells, including the abilities to undergo EMT and the ability of self-renewal. The stepwise process of the budding cancer cell requires significant molecular changes of the cell: for detachment from neoplastic epithelium, dedifferentiation, EMT, cell migration and invasion, and progenitor activity for *de novo* proliferation. Considering that budding CRC cells undergo such a dramatic transient program, probably within a relatively short time frame, the budding CRC cells as an entire cell population constitute a heterogeneous group of cancer cells that possess a strongly modulating molecular profile. In the following I will go through some of the molecular characteristics reported for the budding CRC cells, first cell surface associated proteins and thereafter intracellular molecules. The molecular characteristics involve proteins that participate in regulating differentiation, transcription, translation, cell migration, cell-cell interactions, and adhesion.

2. Laminin-γ2 (Ln-γ2)

The mRNA encoding the Ln-γ2 chain is the first described molecular marker of the budding CRC cells (Pyke et al, 1994). Pyke et al (Pyke et al, 1994) found Ln-γ2 mRNA positive cancer cells in all of 16 colon cancers varying in the presence of positive cells. In a later report, Pyke et al (Pyke et al, 1995) confirmed that the mRNA expression observed in budding colon cancer cells is followed by protein expression in the same cells using a Ln-γ2 specific antibody. Sordat et al (Sordat et al, 1998) reported similar observations in colon cancers using other Ln-γ2 antibody preparations. Laminins are a group of extracellular

glycoproteins being important constituents of basement membrane. They are heterotrimers composed of α, β and γ chains of which there are 5, 4 and 3 isoforms, respectively. The currently used systematic nomenclature defines the composition of a laminin heterotrimers (Aumailley et al, 2005), for example laminin-111 is composed of α1, β1 and γ1 chains and has replaced the earlier used name laminin-1, the most predominant laminin in basement membrane.

The Ln-γ2 chain is only present in the laminin-332 variant, previously known as laminin-5 (Guess & Quaranta, 2009). Laminin-332 links the basement membrane via integrins to hemidesmosomes and thereby stabilizes the polarized positioning of epithelial cell to the basement membrane. Laminins are generally secreted from cells as fully composed heterotrimers, Ln-γ2 being the only exception (Guess & Quaranta, 2009). Ln-γ2 can be secreted as monomer or in complex with the Ln-β3 chain (Guess & Quaranta, 2009) and may have an important significance during budding in CRC (Guess et al, 2009). Pyke et al (Pyke et al, 1995) found Ln-γ2 within the cytoplasm of the budding colon cancer cells and only in rare cases observed basement membrane associated Ln-γ2 immunoreactivity. Sordat et al (Sordat et al, 1998) in contrast found prominent Ln-γ2 immunoreactivity both in the cytoplasm of budding CRC cells and in basement membrane along differentiated tumor cell islands. Today, several monoclonal antibodies against Ln-γ2 are commercially available, some recognize both cytoplasmic and basement membrane associated Ln-γ2, and others are specific for the cytoplasmic precursor form (Hansen et al, 2008; Lindberg et al, 2006). Using Ln-γ2 as marker for budding CRC cells, the antibody employed should therefore be chosen with some consideration.

An interesting finding reported by Sordat et al (Sordat et al, 1998), is that budding cancer cells in addition to express Ln-γ2 also express the Ln-β3 chain, but only at a low level express the Ln-α3 chain. These observations suggest that the secreted Ln-γ2 monomer and Ln-β3-γ2 heterodimer may not function by direct integration into the basement membrane. Other functions have in contrast been found for the secreted Ln-γ2 and Ln-β3 chains. Cell surface directed proteolyic activity performed by matrix metalloproteinase (MMP)-2 (Giannelli et al, 1997) and membrane type-1 (MT1)-MMP (Koshikawa et al, 2000) generates fragments of the Ln-γ2 chain constituting epithelial growth factor (EGF)-like domains that stimulates cell motility. In addition, a cleavage product of Ln-β3 chain generated by MT1-MMP promotes cell migration (Udayakumar et al, 2003). The observations taken together suggest that Ln-γ2 and Ln-β3 chains are contributing to the migratory processes of the budding CRC cells.

In clinical studies, the level of tumor budding in CRC correlated with the level of Ln-γ2 positive budding cells (Shinto et al, 2005). Shinto et al (Shinto et al, 2005) also reported that high-grade Ln-γ2 expression was an independent prognostic indicator, and Aoki et al (Aoki et al, 2002) reported a significant association with synchronous liver metastases. Today, Ln-γ2 expression is considered a strong and potentially clinically applicable prognostic marker that reflects the level of cancer cell budding not only in CRC but also in other cancer types including bladder, esophageal and oral cancer (Guess & Quaranta, 2009).

3. Urokinase Plasminogen Activator Receptor (uPAR)

uPAR (CD87) is a 3-domain highly glycosylated, glycolipid anchored protein. uPAR is a specific high affinity binding receptor for (pro-)uPA, but also binds a number of other

proteins in the extracellular matrix, in particular vitronectin (Eden et al, 2011; Gardsvoll & Ploug, 2007). The glycosyl-phosphatidylinositol (GPI) moiety of uPAR attaches the protein to the outer lipid layer of the cell membrane and allows uPAR to move laterally on cell surfaces and hence rapidly concentrate at focal sites where it mediates its uPA-directed activity and its interaction with vitronectin to the extracellular matrix. Active plasmin is generated from circulating plasminogen on the cell surfaces by a cascade mechanism involving plasmin-mediated conversion of pro-uPA to active uPA. uPA directed plasminogen activation is strongly enhanced after binding of uPA to uPAR on the cell surface (Ploug, 2003; Romer et al, 2004).

The active plasmin enzymatically cleaves or degrades fibrin and fibronectin deposited in the extracellular matrix (ECM), laminins, including the Ln-β3 chain (Goldfinger et al, 1998), L1CAM described below (Mechtersheimer et al, 2001), and activates other matrix degrading protease including MMPs. Through activation of pro-MMPs, MMP-3 (stromelysin-1), MMP-9 (gelatinase-B), MMP-13 (collagenase-3) and MMP-2 (Hald et al, 2011; Juncker-Jensen & Lund, 2011; Monea et al, 2002; Suzuki et al, 2007), plasmin may also mediate degradation of other ECM components including fibrillar collagens. In addition, plasmin activates growth factors like TGF-β (Odekon et al, 1994). Active TGF-β can transform fibroblasts into myofibroblasts (Ronnov-Jessen & Petersen, 1993) and initiate the EMT process in mammary epithelial cells (Fuxe et al, 2010; Thuault et al, 2006). Thus acceleration of the plasminogen activation cascade pathway may elevate the pericellular proteolytic activity and cause dramatic changes in cellular phenotypes.

uPAR was described in budding CRC cells first time in 1994 identified both at the mRNA and protein level (Pyke et al, 1995). Direct comparison with Ln-γ2 expression indicated a strong overlap with Ln-γ2 mRNA (Pyke et al, 1995). Later studies have shown co-expression of uPAR and Ln-γ2 in double immunofluorescence analyses in budding CRC cells (Illemann et al, 2009). uPAR is highly expressed at the invasive front of most CRC not only in budding cancer cells, but also in the complex stromal environment constituting inflammatory cells and myofibroblasts. Activated macrophages located at the invasive front of CRC express high levels of uPAR on the cell surface, hampering an easy discrimination between uPAR-positive budding cancer cells and macrophages. Therefore, to unambiguously identify uPAR-positive budding CRC cells, combining antibodies against uPAR and the epithelial marker cytokeratin (or Ln-γ2) in a double immunofluorescence analysis, would be necessary (Illemann et al, 2009; Romer et al, 2004). uPA mRNA is also expressed in the budding CRC cells (Illemann et al, 2009) indicating that uPAR may function directly through mediation of uPA-directed activities.

In normal colon tissue, uPAR is expressed in a group of differentiated epithelial cells located at the luminal edge of the villi. It has been suggested that uPAR on these cells serve to promote detachment of terminally differentiated colonocytes to be shedded into the colon lumen (Pyke et al, 1994). One may speculate that detachment of budding CRC cells from the main neoplastic glandular structures may mimic the shedding of terminally differentiated cells. In the case of cancer invasion the cancer cells are shed into the tumor stroma. However, this hypothesis does not corroborate with the fact that budding CRC cells show characteristics of dedifferentiation. Nevertheless, this is an interesting interpretation and the invasion of budding CRC cells remain an abnormal process.

Tissue extracts from colon cancers contain highly elevated levels of uPAR compared to the normal tissue, and the high uPAR levels are associated with adverse outcome. uPAR levels

were found to constitute an independent prognostic parameter, importantly being independent of progression stage (Ganesh et al, 1994). A soluble form of uPAR, generated after enzymatic cleavage by uPA or plasmin (Hoyer-Hansen et al, 1997), can be measured in blood, and studies of plasma samples from colon cancer patients substantiate the prognostic significance of uPAR (Stephens et al, 1999; Thurison et al, 2010). As noted above, several different cell types in colon cancers express uPAR, and therefore the prognostic value of uPAR cannot be ascribed solely to the uPAR-positive budding CRC cells. However, in adenocarcinomas, macrophages are by far the predominant uPAR expressing cell type, and therefore most likely account for the elevated levels of uPAR in tumor tissue extracts and in the blood from the cancer patients (Illemann et al, 2009; Romer et al, 2004). uPAR expressing budding cancer cells may nevertheless also contribute to the malignant stage of CRC. uPAR positive cancer cells likely represent a particular malignant cell population since gastric cancer patients with poor prognosis associated with micro-metastatic disease more frequently had uPAR positive cancer cells identified in the bone marrow (Heiss et al, 2002), and uPAR is indeed expressed on invasive cancer cells in gastric cancer (Alpizar-Alpizar et al, 2010).

4. L1 Cell Adhesion Molecule (L1CAM)

L1CAM (L1, CD171) is one of four single-pass trans-membrane proteins forming the group of L1CAM. L1 was first described in 1984 as a neural cell adhesion molecule distinct from the closely related N-CAM group of proteins (Faissner et al, 1984). The other three members of the L1CAM family are NrCAM, CHL1 and neurofascin. All four genes are thoroughly characterized and highly expressed in the nervous system (Chen & Zhou, 2010). The L1CAMs contain 6 immunoglobulin-like motifs and 4 or 5 fibronectin type III repeats. The Cytoplasmic domain allows binding to cytoskeletal ankyrin and ERM proteins (ezrin-radixin-moesin) that associates with actin filaments (Bretscher et al, 2002). L1 is involved in cell-cell adhesion by homophilic interactions, cell-ECM interactions and cell surface interactions by binding integrins and can directly mediate cytoskeletal changes and signal transduction through its cytoplasmic domain (Chen & Zhou, 2010; Kadmon & Altevogt, 1997; Schmid & Maness, 2008). L1 is highly expressed in normal and diseased brain, and plays a critical role for development and organization of neuronal cell groups (Demyanenko et al, 2001; Sakurai et al, 2001). L1 has functions overlapping with NrCAM identified in double-deficient mice that show postnatal lethality in contrast to the corresponding single deficient mice (Sakurai et al, 2001). L1 has been reported to mediate cell adhesion and transendothelial migration also of dendritic cells (Maddaluno et al, 2009).

Expression of L1 in budding CRC cells has been shown both at the protein (Gavert et al, 2005; Kajiwara et al, 2011) and the mRNA level (Kajiwara et al, 2011). Gavert et al (Gavert et al, 2005) found L1 positive budding cancer cells in 68% of 19 colon cancer cases studied. Kajiwara et al (Kajiwara et al, 2011) studied 275 cases of CRC and also found L1 expression at the invasive front. Furthermore the authors showed that the L1 expression increased according to the grade of tumor budding and that L1 expression was correlated with nodal involvement both at the protein and mRNA level. In normal colon mucosa, L1 is expressed on sporadic intramucosal nerve axons and L1 positive enteric nerve axons were found in the deeper layers of the bowel wall (Gavert et al, 2005). In fact, the authors also noted that L1-positive colon cancer cells invaded along L1 positive nerve axons, and suggested that L1-mediated adhesive interactions between the two cell populations may facilitate the invasion

of cancer cells. In this connection, it is curious that also uPAR can be found in enteric nerve bundles (Laerum et al, 2008). It is also interesting to note that L1 has been detected in human and murine myeloid and lymphoid cells (Ebeling et al, 1996; Kowitz et al, 1992) and that no L1 immunoreactivity has been reported in tumor stroma of the CRC. Whether the level of L1 in these cells is below the detection limit, is lost by proteolytic shedding, transcriptional or translational downregulation (Hubbe et al, 1993) remains to be clarified.

Proteolytic shedding of the L1 ectodomain has been reported to be performed by the disintegrin and metalloproteinases (ADAM) ADAM10 (Gutwein et al, 2003; Maretzky et al, 2005; Mechtersheimer et al, 2001), ADAM17 (Maretzky et al, 2005), and plasmin (Mechtersheimer et al, 2001). Shedding of the L1 ectodomain will prevent cell-cell interactions and binding interactions with other cell surface proteins and matrix components affecting the biochemical functions of the protein. Cleavage of L1 by ADAM10 releases an approximately 200kDa L1 fragment that can promote cell migration (Gutwein et al, 2003; Maretzky et al, 2005; Mechtersheimer et al, 2001). Similar activity was reported for the plasmin released L1 fragment (Mechtersheimer et al, 2001). In this connection it is important to note that ADAM10 immunoreactivity has been reported to be co-expressed in budding CRC cells (Gavert et al, 2005), and that cleavage can occur to an extent that a soluble L1 fragment can be measured in serum from cancer patients (Fogel et al, 2003). L1 expression in budding CRC cells may therefore be involved in at least 3 different processes during CRC cell budding: detachment from the differentiated neoplastic glandular structures, cell-cell interactions with L1-positive nerve axons or dendritic cells as well as in the contribution to CRC cell migration upon extracellular enzymatic processing.

5. Matrix Metalloproteinase 7 (MMP-7)

A number of other genes have been found to be focally expressed in the budding colon cancer cells. I have already mentioned some of the MMPs in connection with Ln-γ2 processing and plasmin-activated MMPs. MMP-7 (matrilysin-1) belongs to the group of matrix metalloproteinases (MMPs) (Das et al, 2003; Folgueras et al, 2004), which are zinc dependent endopeptidases known for their ability to cleave several ECM proteins. The activity of MMPs is regulated at the level of pro-MMP conversion and blocking by specific tissue inhibitors of metalloproteinases (TIMP) of which 4 are known (Nagase et al, 2006). MMP-7 can digest several ECM proteins including elastin, collagen IV and vitronectin (Ii et al, 2006). In addition, MMP7 has been shown to play important roles in the regulation of a variety of biochemical processes, such as the activation of MMP-2 and MMP-9 and shedding of Fas-ligand, pro-tumor necrosis factor-α, and E-cadherin (Curino et al, 2004; Ii et al, 2006; Nagase et al, 2006). MMP-7 itself is activated by other endoproteinases including plasmin and trypsin. MMP-7 was recently shown to bind and cleave the Ln-β3 chain. The cleaved Ln-β3 fragments was found to mediate cell migration (Remy et al, 2006). MMP-7 immunoreactivity has been reported in budding CRC cancer cells (Kurokawa et al, 2005; Masaki et al, 2001) and was found to co-localize with laminin-5 and Ln-β3 chain in human xenografted colon tumors (Remy et al, 2006). In the relatively small prognostic study by Masaki et al (Masaki et al, 2001), including 38 patients with early CRC, scoring the MMP-7 immunoreactive budding cancer cells was linked to distant metastasis and adverse outcome. However, MMP-7 immunoreactivity was seen in less than half of the cases with moderate to severe budding as determined on H&E stained sections, indicating that MMP-7 is not

consistently expressed in budding CRC. In a study of 494 CRC cases, MMP-7 mRNA was found to be an independent risk factor predicting nodal metastasis (Kurokawa et al, 2005). In the context of budding CRC cells, the ability of MMP-7 to shed E-cadherin and cleave the Ln-β3 chain into a motility stimulating fragment suggest that MMP-7 takes part in the early steps involving detachment from the glandular structures and mobilizing cancer cell migration.

6. Membrane Type-1 Matrix Metalloproteinase (MT1-MMP)

MT1-MMP (MMP-14) immunoreactivity has also been demonstrated in budding CRC cells (Hlubek et al, 2004). MT1-MMP belongs to the group of membrane-bound MMPs and is a trans-membrane protein capable of cleaving fibrillar collagen. This MMP is essential for normal development (Holmbeck et al, 1999). MT1-MMP binds extracellular MMP-2 and TIMP-2 into a ternary complex that is important for the regulation of enzymatic activity on the cell surface (Sato & Takino, 2010; Strongin, 2010). The active enzyme can also cleave a number of other membrane-associated proteins including pro-tumor necrosis factor-α and CD44 (Folgueras et al, 2004). Expression of MT1-MMP mRNA is prominent in colon cancer associated stroma (Okada et al, 1995). As also noted above for evaluation of uPAR expression, strong stromal MT1-MMP expression in the invasive front area will prevent unambiguous identification of the protein in budding cancer cells. Expression of MT1-MMP on the surface of budding CRC cells would allow significant surface associated proteolysis either directly or through activation of MMP-2, which together with TIMP-2 would be provided by adjacent stromal cells (Holten-Andersen et al, 2005; Okada et al, 1995; Poulsom et al, 1992). As already mentioned, MT1-MMP can cleave Ln-γ2 alone or in cooperation with MMP-2, which results in a Ln-γ2 fragment with capacity to stimulate migration (Koshikawa et al, 2005; Koshikawa et al, 2004). More immunohistochemical studies are needed to better clarify the expression patterns of MT1-MMP in budding CRC cells.

The activities of the above mentioned proteins Ln-γ2, uPAR, L1, MMP-7 and MT1-MMP are all taking place on the cell surface and pericellular matrix. The following molecules will be related to intracellular acitivities.

7. β-catenin (Wnt pathway)

β-catenin is an important intracellular protein to consider in the characterization of budding CRC cells. Nuclear β-catenin activates transcription of a number of genes including those encoding Ln-γ2, uPAR and MMP-7 (Brabletz et al, 2004; Crawford et al, 1999; Hlubek et al, 2001; Mann et al, 1999). β-catenin is a protein that binds the cytoplasmic domain of E-cadherin and by concomitantly binding actin filaments contributes to maintain the cytoskeleton and the epithelial integrity. The intracellular localization of β-catenin is linked to the status of Wnt activity directed through the trans-membrane receptor Frizzled. Cellular activation by Wnt cause trans-localization of β-catenin to the nuclei, which through binding to transcription factors, affects transcription. β-catenin is expressed both in the differentiated glandular structures and in the budding CRC cells, but the localization changes from cytoplasmic to nuclear. In the case of budding CRC cells, β-catenin is seen in the nuclei (Brabletz et al, 2001; Brabletz et al, 2004; Gavert et al, 2005; Gavert et al, 2011; Hlubek et al, 2001; Jass et al, 2003). Translocation of β-catenin to the nuclei has been used as

a reference marker for CRC cell budding to show co-expression of Ln-γ2 (Hlubek et al, 2001), L1 (Gavert et al, 2011), and disruption of E-cadherin (Brabletz et al, 2001). The EMT process and stem cell characteristics has been associated with the activity of β-catenin in cooperation with TGF-β (Brabletz et al, 2005; Fuxe et al, 2010). For a further discussion on signal transduction in budding CRC cells I suggest to consult the review by Prall (Prall, 2007).

8. Hepatocyte growth factor activator inhibitor type 2-Related Small Peptide (H2RSP)

An interesting novel protein identified to be specifically up-regulated in connection with CRC cell budding is hepatocyte growth factor activator inhibitor type 2-related small peptide (H2RSP) (Uchiyama et al, 2007). The H2RSP gene was first described in 2001 by Itoh et al (Itoh et al, 2001), who observed H2RSP expression in tissues obtained from the gastrointestinal tract. H2RSP protein, which is identical to immortalization-upregulated protein (IMUP-1), is a small protein constituting 106 amino acids. Its interaction partner(s) and function(s) remains to be established. An interaction with single stranded G-rich DNA probably via a lysine-rich domain of the protein was discussed to be involved in nuclear translocalization (Uchiyama et al, 2007). Uchiyama et al (Uchiyama et al, 2007) found by immunohistochemistry that H2RSP was located in the cytoplasm of normal un-differentiated epithelial cells in the colon, but found a change in localization to the nuclei in the differentiated epithelial cells. The authors suggested that H2RSP is involved in the transition process from the proliferation phase to terminal differentiation of intestinal epithelium. Looking at colon tumors, they found H2RSP immunoreactivity to be fully lost in the central differentiated tumor areas, but focally upregulated in the invasive front, including in budding CRC cells. In these cells, the H2RSP staining was located in the cytoplasm. The expression of H2RSP coincided with nuclear localization of β-catenin, focal co-expression of p16 and focal loss of proliferation marker Ki67. Thus, H2RSP, as confined to the epithelial cell population, is an interesting marker of budding CRC indicating a stage of dedifferentiation and growth arrest. A potential function in the related hepatocyte growth factor/scatter factor signaling pathway through Met receptor, which is taking place at the cell surface, seems unlikely so far.

9. microRNA-21 (miR-21)

MicroRNAs (miRNA) constitute a group of short, 18-23 base-pair long, non-coding RNAs. MiRNAs are processed from precursor RNA transcripts into mature active forms by a mechanism only partially understood. A generally accepted sequence of steps for the biochemical processing of precursor miRNA to the mature forms is known as the ‘‘linear” canonical pathway (Winter et al, 2009). MiRNAs have been found to play particular important roles in cell differentiation by negatively regulating translation (Calin & Croce, 2009; Iorio & Croce, 2009; Lim et al, 2005; Liu & Olson, 2010). miRNAs bind to specific 3’UTR sequences of mRNAs and thereby prevent efficient translation or mediate degradation of target mRNAs. For identification of miRNAs in tissue sections, *in situ* hybridization is an indispensable technique, which different from mRNA *in situ* hybridization cannot be replaced by immunohistochemistry. Specific detection of miRNA *in situ* therefore sets high requirements to the detection probes. Here LNA:DNA chimeric oligo

probes have shown to fulfill at least some of the requirement for sufficient specificity and sensitivity (Jorgensen et al, 2010; Kloosterman et al, 2006). In an *in situ* hybridization study of miR-21 expression in stage II CRC, expression of miR-21 was seen in some budding CRC cell located at the invasive front of the tumors (Nielsen et al, 2011). A clear identification and quantitative estimation of the miR-21 positive budding cancer cells was however confounded by high miR-21 signal also in the tumor stroma. Therefore further studies are needed to better address the association of miR-21 to budding CRC cells, including double fluorescence labeling as also discussed above for uPAR. In this case, *in situ* hybridization and immunohistochemistry should be combined as exemplified by Sempere et al (Sempere et al, 2010), who applied this technology in routinely processed clinical paraffin samples.

miR-21 is highly upregulated in CRC compared to normal colon tissue (Nielsen et al, 2011; Schetter et al, 2008) and high expression is linked to adverse outcome in stage II CRC (Nielsen et al, 2011; Schetter et al, 2008). The mechanisms of action of miR-21 in budding cancer cells and in cancer progression in general are unclear. In mice lacking miR-21 (Ma et al, 2011) the number of chemically induced skin tumors was significantly lower than in wild type mice. In keratinocytes from the miR-21 deficient mice, increased expression of SPRY1, PTEN and PDCD4 was found, which is consistent with findings of miR-21 target genes in different human cell lines: *Spry1* in cardiac fibroblasts (Thum et al, 2008), the tumor suppressor *Pten* in hepatocytes and cardiac fibroblasts (Meng et al, 2007; Roy et al, 2009), and the tumor suppressor *Pdcd4* in a variety of cell lines (Asangani et al, 2008; Talotta et al, 2009). In budding CRC cells, miR-21 may suppress the expression level of PTEN and PDCD4 and thereby prevent cell death. miR-21 has also been attributed a central role in TGF-β induced EMT (Zavadil et al, 2007). Whether these regulatory events occur at the invasive front and in budding cancer cells remain to be established.

10. p16, Ki67 and cdx2

In connection with the studies mentioned above a couple of other proteins have been reported to focally change expression pattern in the budding CRC cells. These include p16 (Jass et al, 2003; Uchiyama et al, 2007) and cdx2 (Brabletz et al, 2004). The tumor suppressor p16 plays an important role in regulation of the cell cycle through interaction with p53 and as an inhibitor of cyclin dependent kinase 4 (CDK4). Both Uchiyama et al (Uchiyama et al, 2007) and Jass et al (Jass et al, 2003) noted that p16 was strongly expressed as a cytoplasmic immunoreactivity in the budding CRC cells. The increased expression may block translocation of CDK4 to the nuclei and thereby increase cyclin D1 levels and reduce proliferation (Jass et al, 2003). In fact, the proliferation marker Ki67 is lost in budding CRC cells (Brabletz et al, 2001; Uchiyama et al, 2007). The homeobox *Cdx2* encodes an intestine-specific transcription factor and is considered a tumor suppressor. Cdx2 immunoreactivity was absent in budding CRC cells in contrast to nuclei-related staining in the more differentiated tumor areas (Brabletz et al, 2004). It is intriguing that p16 and Cdx2, as so-called tumor suppressors, present themselves differently with respect to their presence in these highly malignant cells.

Although positive markers of the budding CRC cells provide clues to a mechanistic interpretation, loss of expression or intracellular translocation may provide significant help to characterize the budding process as well. As a final comment, I would like to suggest

including the following 3 proteins as reference markers for the dedifferentiated budding CRC cells: Ln-γ2 giving a positive reaction in the cytoplasm, β-catenin giving a positive reaction in the nuclei, and Ki67 being negative (in constrast to the neighboring cancer cells). These markers will complement each other sufficiently as a reference profile and well-characterized antibodies are commercially available and applicable in paraffin embedded specimens.

11. Conclusion

A successful budding CRC cell encounters dramatic challenges during the short local expedition: dedifferentiation and detachment from the established differentiated glandular structure, migration through a foreign stromal tissue rich in inflammatory cells and desmoplastic cells, settlement in the new stromal environment and re-initiation of its own proliferation program. In this chapter I have reviewed current literature in order to compile the molecular traits linked to these cells and put the proteins' function into the budding processes. I propose that budding-initiating factors, such as TGF-β and Wnts, derived from in the stromal environment in the tumor periphery, and that the factors are received by cancer cells with progenitor capacity and which are able to address an activation program involving β-catenin mediated dedifferentiation and migration. From the compiled data, the following sequence of steps could be anticipated: increased levels of ECM degrading proteases, mediated by uPAR/uPA and MT1-MMP/MMP2, and MMP7 taking care of E-cadherin processing. The proliferation program is halted. This could be followed by a discrete EMT program, which transiently changes the morphology of the cells and allows detachment and budding. Upregulation and secretion of Ln-β3 and Ln-γ2 and subsequent extracellular processing will result in strongly motility-inducing fragments that allow migration through the stromal tissue. At the same time L1 is introduced changing the preferred cellular interaction partners to neuronal axons and dendritic cells as well as introducing yet other migration stimulating factors: the ADAM10 and plasmin processed L1 ectodoamins. Budding cancer cells invade as distant as possible into the stroma. One mechanism that could make the invasive cells stop and settle in the stromal environment could be a change in balance between active proteases and protease inhibitors, which may be shifted in favor of the inhibitors. Both PAI-1 and TIMPs are highly expressed in stromal cells at the invasive front (Holten-Andersen et al, 2005; Illemann et al, 2004; Poulsom et al, 1992). Increased protease inhibitor levels would prevent the formation of migration stimulating laminin fragments and also the surface associated proteases needed for invasion through the ECM. After settlement the excess laminins will be deposited and may contribute to form a loose basement membrane for the cancer cell to stick to and polarize, L1 will again be replaced by E-cadherin and proliferation and differentiation programs are reinitiated.

12. References

Alpizar-Alpizar, W., Nielsen, B. S., Sierra, R., Illemann, M., Ramirez, J. A., Arias, A., Duran, S., Skarstein, A., Ovrebo, K., Lund, L. R. & Laerum, O. D. (2010). Urokinase plasminogen activator receptor is expressed in invasive cells in gastric carcinomas from high- and low-risk countries. *Int. J. Cancer*, Vol. 126, No. 2, pp. 405-415.

Aoki, S., Nakanishi, Y., Akimoto, S., Moriya, Y., Yoshimura, K., Kitajima, M., Sakamoto, M. & Hirohashi, S. (2002). Prognostic significance of laminin-5 gamma2 chain expression in colorectal carcinoma: immunohistochemical analysis of 103 cases. *Dis. Colon Rectum*, Vol. 45, No. 11, pp. 1520-1527.

Asangani, I. A., Rasheed, S. A., Nikolova, D. A., Leupold, J. H., Colburn, N. H., Post, S. & Allgayer, H. (2008). MicroRNA-21 (miR-21) post-transcriptionally downregulates tumor suppressor Pdcd4 and stimulates invasion, intravasation and metastasis in colorectal cancer. *Oncogene*, Vol. 27, No. 15, pp. 2128-2136.

Aumailley, M., Bruckner-Tuderman, L., Carter, W. G., Deutzmann, R., Edgar, D., Ekblom, P., Engel, J., Engvall, E., Hohenester, E., Jones, J. C., Kleinman, H. K., Marinkovich, M. P., Martin, G. R., Mayer, U., Meneguzzi, G., Miner, J. H., Miyazaki, K., Patarroyo, M., Paulsson, M., Quaranta, V., Sanes, J. R., Sasaki, T., Sekiguchi, K., Sorokin, L. M., Talts, J. F., Tryggvason, K., Uitto, J., Virtanen, I., von der, M. K., Wewer, U. M., Yamada, Y. & Yurchenco, P. D. (2005). A simplified laminin nomenclature. *Matrix Biol.*, Vol. 24, No. 5, pp. 326-332.

Brabletz, T., Hlubek, F., Spaderna, S., Schmalhofer, O., Hiendlmeyer, E., Jung, A. & Kirchner, T. (2005). Invasion and metastasis in colorectal cancer: epithelial-mesenchymal transition, mesenchymal-epithelial transition, stem cells and beta-catenin. *Cells Tissues. Organs*, Vol. 179, No. 1-2, pp. 56-65.

Brabletz, T., Jung, A., Reu, S., Porzner, M., Hlubek, F., Kunz-Schughart, L. A., Knuechel, R. & Kirchner, T. (2001). Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc. Natl. Acad. Sci. U. S. A*, Vol. 98, No. 18, pp. 10356-10361.

Brabletz, T., Spaderna, S., Kolb, J., Hlubek, F., Faller, G., Bruns, C. J., Jung, A., Nentwich, J., Duluc, I., Domon-Dell, C., Kirchner, T. & Freund, J. N. (2004). Down-regulation of the homeodomain factor Cdx2 in colorectal cancer by collagen type I: an active role for the tumor environment in malignant tumor progression. *Cancer Res.*, Vol. 64, No. 19, pp. 6973-6977.

Bretscher, A., Edwards, K. & Fehon, R. G. (2002). ERM proteins and merlin: integrators at the cell cortex. *Nat. Rev. Mol. Cell Biol.*, Vol. 3, No. 8, pp. 586-599.

Calin, G. A. & Croce, C. M. (2009). Chronic lymphocytic leukemia: interplay between noncoding RNAs and protein-coding genes. *Blood*, Vol. 114, No. 23, pp. 4761-4770.

Chaffer, C. L. & Weinberg, R. A. (2011). A perspective on cancer cell metastasis. *Science*, Vol. 331, No. 6024, pp. 1559-1564.

Chen, L. & Zhou, S. (2010). "CRASH"ing with the worm: insights into L1CAM functions and mechanisms. *Dev. Dyn.*, Vol. 239, No. 5, pp. 1490-1501.

Crawford, H. C., Fingleton, B. M., Rudolph-Owen, L. A., Goss, K. J., Rubinfeld, B., Polakis, P. & Matrisian, L. M. (1999). The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. *Oncogene*, Vol. 18, No. 18, pp. 2883-2891.

Curino, A., Patel, V., Nielsen, B. S., Iskander, A. J., Ensley, J. F., Yoo, G. H., Holsinger, F. C., Myers, J. N., El-Nagaar, A., Kellman, R. M., Shillitoe, E. J., Molinolo, A. A., Gutkind, J. S. & Bugge, T. H. (2004). Detection of plasminogen activators in oral cancer by laser capture microdissection combined with zymography. *Oral Oncol.*, Vol. 40, No. 10, pp. 1026-1032.

Das, S., Mandal, M., Chakraborti, T., Mandal, A. & Chakraborti, S. (2003). Structure and evolutionary aspects of matrix metalloproteinases: a brief overview. *Mol. Cell Biochem.*, Vol. 253, No. 1-2, pp. 31-40.

Demyanenko, G. P., Shibata, Y. & Maness, P. F. (2001). Altered distribution of dopaminergic neurons in the brain of L1 null mice. *Brain Res. Dev. Brain Res.*, Vol. 126, No. 1, pp. 21-30.

Ebeling, O., Duczmal, A., Aigner, S., Geiger, C., Schollhammer, S., Kemshead, J. T., Moller, P., Schwartz-Albiez, R. & Altevogt, P. (1996). L1 adhesion molecule on human lymphocytes and monocytes: expression and involvement in binding to alpha v beta 3 integrin. *Eur. J. Immunol.*, Vol. 26, No. 10, pp. 2508-2516.

Eden, G., Archinti, M., Furlan, F., Murphy, R. & Degryse, B. (2011). The Urokinase Receptor Interactome. *Curr. Pharm. Des*. Epub ahead of print.

Faissner, A., Kruse, J., Goridis, C., Bock, E. & Schachner, M. (1984). The neural cell adhesion molecule L1 is distinct from the N-CAM related group of surface antigens BSP-2 and D2. *EMBO J.*, Vol. 3, No. 4, pp. 733-737.

Fogel, M., Gutwein, P., Mechtersheimer, S., Riedle, S., Stoeck, A., Smirnov, A., Edler, L., Ben-Arie, A., Huszar, M. & Altevogt, P. (2003). L1 expression as a predictor of progression and survival in patients with uterine and ovarian carcinomas. *Lancet*, Vol. 362, No. 9387, pp. 869-875.

Folgueras, A. R., Pendas, A. M., Sanchez, L. M. & Lopez-Otin, C. (2004). Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *Int. J. Dev. Biol.*, Vol. 48, No. 5-6, pp. 411-424.

Fujimori, T., Fujii, S., Saito, N. & Sugihara, K. (2009). Pathological diagnosis of early colorectal carcinoma and its clinical implications. *Digestion*, Vol. 79 Suppl 1, pp. 40-51.

Fuxe, J., Vincent, T. & Garcia de, H. A. (2010). Transcriptional crosstalk between TGF-beta and stem cell pathways in tumor cell invasion: role of EMT promoting Smad complexes. *Cell Cycle*, Vol. 9, No. 12, pp. 2363-2374.

Gabbert, H., Wagner, R., Moll, R. & Gerharz, C. D. (1985). Tumor dedifferentiation: an important step in tumor invasion. *Clin. Exp. Metastasis*, Vol. 3, No. 4, pp. 257-279.

Ganesh, S., Sier, C. F., Heerding, M. M., Griffioen, G., Lamers, C. B. & Verspaget, H. W. (1994). Urokinase receptor and colorectal cancer survival. *Lancet*, Vol. 344, No. 8919, pp. 401-402.

Gardsvoll, H. & Ploug, M. (2007). Mapping of the vitronectin-binding site on the urokinase receptor: involvement of a coherent receptor interface consisting of residues from both domain I and the flanking interdomain linker region. *J. Biol. Chem.*, Vol. 282, No. 18, pp. 13561-13572.

Gavert, N., Conacci-Sorrell, M., Gast, D., Schneider, A., Altevogt, P., Brabletz, T. & Ben-Ze'ev, A. (2005). L1, a novel target of beta-catenin signaling, transforms cells and is expressed at the invasive front of colon cancers. *J. Cell Biol.*, Vol. 168, No. 4, pp. 633-642.

Gavert, N., Vivanti, A., Hazin, J., Brabletz, T. & Ben-Ze'ev, A. (2011). L1-mediated colon cancer cell metastasis does not require changes in EMT and cancer stem cell markers. *Mol. Cancer Res.*, Vol. 9, No. 1, pp. 14-24.

Giannelli, G., Falk-Marzillier, J., Schiraldi, O., Stetler-Stevenson, W. G. & Quaranta, V. (1997). Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. *Science*, Vol. 277, No. 5323, pp. 225-228.

Goldfinger, L. E., Stack, M. S. & Jones, J. C. (1998). Processing of laminin-5 and its functional consequences: role of plasmin and tissue-type plasminogen activator. *J. Cell Biol.*, Vol. 141, No. 1, pp. 255-265.

Guess, C. M., Lafleur, B. J., Weidow, B. L. & Quaranta, V. (2009). A decreased ratio of laminin-332 beta3 to gamma2 subunit mRNA is associated with poor prognosis in colon cancer. *Cancer Epidemiol. Biomarkers Prev.*, Vol. 18, No. 5, pp. 1584-1590.

Guess, C. M. & Quaranta, V. (2009). Defining the role of laminin-332 in carcinoma. *Matrix Biol.*, Vol. 28, No. 8, pp. 445-455.

Gutwein, P., Mechtersheimer, S., Riedle, S., Stoeck, A., Gast, D., Joumaa, S., Zentgraf, H., Fogel, M. & Altevogt, D. P. (2003). ADAM10-mediated cleavage of L1 adhesion molecule at the cell surface and in released membrane vesicles. *FASEB J.*, Vol. 17, No. 2, pp. 292-294.

Hald, A., Rono, B., Melander, M. C., Ding, M., Holck, S. & Lund, L. R. (2011). MMP9 is protective against lethal inflammatory mass lesions in the mouse colon. *Dis. Model. Mech.*, Vol. 4, No. 2, pp. 212-227.

Hansen, L. V., Laerum, O. D., Illemann, M., Nielsen, B. S. & Ploug, M. (2008). Altered expression of the urokinase receptor homologue, C4.4A, in invasive areas of human esophageal squamous cell carcinoma. *Int. J. Cancer*, Vol. 122, No. 4, pp. 734-741.

Hase, K., Shatney, C., Johnson, D., Trollope, M. & Vierra, M. (1993). Prognostic value of tumor "budding" in patients with colorectal cancer. *Dis. Colon Rectum*, Vol. 36, No. 7, pp. 627-635.

Heiss, M. M., Simon, E. H., Beyer, B. C., Gruetzner, K. U., Tarabichi, A., Babic, R., Schildberg, F. W. & Allgayer, H. (2002). Minimal residual disease in gastric cancer: evidence of an independent prognostic relevance of urokinase receptor expression by disseminated tumor cells in the bone marrow. *J. Clin. Oncol.*, Vol. 20, No. 8, pp. 2005-2016.

Hlubek, F., Jung, A., Kotzor, N., Kirchner, T. & Brabletz, T. (2001). Expression of the invasion factor laminin gamma2 in colorectal carcinomas is regulated by beta-catenin. *Cancer Res.*, Vol. 61, No. 22, pp. 8089-8093.

Hlubek, F., Spaderna, S., Jung, A., Kirchner, T. & Brabletz, T. (2004). Beta-catenin activates a coordinated expression of the proinvasive factors laminin-5 gamma2 chain and MT1-MMP in colorectal carcinomas. *Int. J. Cancer*, Vol. 108, No. 2, pp. 321-326.

Holmbeck, K., Bianco, P., Caterina, J., Yamada, S., Kromer, M., Kuznetsov, S. A., Mankani, M., Robey, P. G., Poole, A. R., Pidoux, I., Ward, J. M. & Birkedal-Hansen, H. (1999). MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell*, Vol. 99, No. 1, pp. 81-92.

Holten-Andersen, M. N., Hansen, U., Brunner, N., Nielsen, H. J., Illemann, M. & Nielsen, B. S. (2005). Localization of tissue inhibitor of metalloproteinases 1 (TIMP-1) in human colorectal adenoma and adenocarcinoma. *Int. J. Cancer*, Vol. 113, No. 2, pp. 198-206.

Hoyer-Hansen, G., Ploug, M., Behrendt, N., Ronne, E. & Dano, K. (1997). Cell-surface acceleration of urokinase-catalyzed receptor cleavage. *Eur. J. Biochem.*, Vol. 243, No. 1-2, pp. 21-26.

Hubbe, M., Kowitz, A., Schirrmacher, V., Schachner, M. & Altevogt, P. (1993). L1 adhesion molecule on mouse leukocytes: regulation and involvement in endothelial cell binding. *Eur. J. Immunol.*, Vol. 23, No. 11, pp. 2927-2931.

Ii, M., Yamamoto, H., Adachi, Y., Maruyama, Y. & Shinomura, Y. (2006). Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. *Exp. Biol. Med. (Maywood.)*, Vol. 231, No. 1, pp. 20-27.

Illemann, M., Bird, N., Majeed, A., Laerum, O. D., Lund, L. R., Dano, K. & Nielsen, B. S. (2009). Two distinct expression patterns of urokinase, urokinase receptor and plasminogen activator inhibitor-1 in colon cancer liver metastases. *Int. J. Cancer*, Vol. 124, No. 8, pp. 1860-1870.

Illemann, M., Hansen, U., Nielsen, H. J., Andreasen, P. A., Hoyer-Hansen, G., Lund, L. R., Dano, K. & Nielsen, B. S. (2004). Leading-edge myofibroblasts in human colon cancer express plasminogen activator inhibitor-1. *Am. J. Clin. Pathol.*, Vol. 122, No. 2, pp. 256-265.

Iorio, M. V. & Croce, C. M. (2009). MicroRNAs in cancer: small molecules with a huge impact. *J. Clin. Oncol.*, Vol. 27, No. 34, pp. 5848-5856.

Itoh, H., Kataoka, H., Yamauchi, M., Naganuma, S., Akiyama, Y., Nuki, Y., Shimomura, T., Miyazawa, K., Kitamura, N. & Koono, M. (2001). Identification of hepatocyte growth factor activator inhibitor type 2 (HAI-2)-related small peptide (H2RSP): its nuclear localization and generation of chimeric mRNA transcribed from both HAI-2 and H2RSP genes. *Biochem. Biophys. Res. Commun.*, Vol. 288, No. 2, pp. 390-399.

Jass, J. R., Barker, M., Fraser, L., Walsh, M. D., Whitehall, V. L., Gabrielli, B., Young, J. & Leggett, B. A. (2003). APC mutation and tumour budding in colorectal cancer. *J. Clin. Pathol.*, Vol. 56, No. 1, pp. 69-73.

Jorgensen, S., Baker, A., Moller, S. & Nielsen, B. S. (2010). Robust one-day in situ hybridization protocol for detection of microRNAs in paraffin samples using LNA probes. *Methods*, Vol. 52, No. 4, pp. 375-381.

Juncker-Jensen, A. & Lund, L. R. (2011). Phenotypic overlap between MMP-13 and the plasminogen activation system during wound healing in mice. *PLoS. One.*, Vol. 6, No. 2, pp. e16954.

Kadmon, G. & Altevogt, P. (1997). The cell adhesion molecule L1: species- and cell-type-dependent multiple binding mechanisms. *Differentiation*, Vol. 61, No. 3, pp. 143-150.

Kajiwara, Y., Ueno, H., Hashiguchi, Y., Shinto, E., Shimazaki, H., Mochizuki, H. & Hase, K. (2011). Expression of l1 cell adhesion molecule and morphologic features at the invasive front of colorectal cancer. *Am. J. Clin. Pathol.*, Vol. 136, No. 1, pp. 138-144.

Kirkland, S. C., (2009). Type I collagen inhibits differentiation and promotes a stem cell-like phenotype in human colorectal carcinoma cells. *Br. J. Cancer*, Vol. 101, No. 2, pp. 320-326.

Kloosterman, W. P., Wienholds, E., de, B. E., Kauppinen, S. & Plasterk, R. H. (2006). In situ detection of miRNAs in animal embryos using LNA-modified oligonucleotide probes. *Nat. Methods*, Vol. 3, No. 1, pp. 27-29.

Koshikawa, N., Giannelli, G., Cirulli, V., Miyazaki, K. & Quaranta, V. (2000). Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5. *J. Cell Biol.*, Vol. 148, No. 3, pp. 615-624.

Koshikawa, N., Minegishi, T., Sharabi, A., Quaranta, V. & Seiki, M. (2005). Membrane-type matrix metalloproteinase-1 (MT1-MMP) is a processing enzyme for human laminin gamma 2 chain. *J. Biol. Chem.*, Vol. 280, No. 1, pp. 88-93.

Koshikawa, N., Schenk, S., Moeckel, G., Sharabi, A., Miyazaki, K., Gardner, H., Zent, R. & Quaranta, V. (2004). Proteolytic processing of laminin-5 by MT1-MMP in tissues and its effects on epithelial cell morphology. *FASEB J.*, Vol. 18, No. 2, pp. 364-366.

Kowitz, A., Kadmon, G., Eckert, M., Schirrmacher, V., Schachner, M. & Altevogt, P. (1992). Expression and function of the neural cell adhesion molecule L1 in mouse leukocytes. *Eur. J. Immunol.*, Vol. 22, No. 5, pp. 1199-1205.

Kurokawa, S., Arimura, Y., Yamamoto, H., Adachi, Y., Endo, T., Sato, T., Suga, T., Hosokawa, M., Shinomura, Y. & Imai, K. (2005). Tumour matrilysin expression predicts metastatic potential of stage I (pT1) colon and rectal cancers. *Gut*, Vol. 54, No. 12, pp. 1751-1758.

Laerum, O. D., Illemann, M., Skarstein, A., Helgeland, L., Ovrebo, K., Dano, K. & Nielsen, B. S. (2008). Crohn's disease but not chronic ulcerative colitis induces the expression of PAI-1 in enteric neurons. *Am. J. Gastroenterol.*, Vol. 103, No. 9, pp. 2350-2358.

Lim, L. P., Lau, N. C., Garrett-Engele, P., Grimson, A., Schelter, J. M., Castle, J., Bartel, D. P., Linsley, P. S. & Johnson, J. M. (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature*, Vol. 433, No. 7027, pp. 769-773.

Lindberg, P., Larsson, A. & Nielsen, B. S. (2006). Expression of plasminogen activator inhibitor-1, urokinase receptor and laminin gamma-2 chain is an early coordinated event in incipient oral squamous cell carcinoma. *Int. J. Cancer*, Vol. 118, No. 12, pp. 2948-2956.

Liu, N. & Olson, E. N. (2010). MicroRNA regulatory networks in cardiovascular development. *Dev. Cell*, Vol. 18, No. 4, pp. 510-525.

Ma, X., Kumar, M., Choudhury, S. N., Becker Buscaglia, L. E., Barker, J. R., Kanakamedala, K., Liu, M. F. & Li, Y. (2011). Loss of the miR-21 allele elevates the expression of its target genes and reduces tumorigenesis. *Proc. Natl. Acad. Sci. U. S. A*, Vol. 108, No. 25, pp. 10144-10149.

Maddaluno, L., Verbrugge, S. E., Martinoli, C., Matteoli, G., Chiavelli, A., Zeng, Y., Williams, E. D., Rescigno, M. & Cavallaro, U. (2009). The adhesion molecule L1 regulates transendothelial migration and trafficking of dendritic cells. *J. Exp. Med.*, Vol. 206, No. 3, pp. 623-635.

Mann, B., Gelos, M., Siedow, A., Hanski, M. L., Gratchev, A., Ilyas, M., Bodmer, W. F., Moyer, M. P., Riecken, E. O., Buhr, H. J. & Hanski, C. (1999). Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *Proc. Natl. Acad. Sci. U. S. A*, Vol. 96, No. 4, pp. 1603-1608.

Maretzky, T., Schulte, M., Ludwig, A., Rose-John, S., Blobel, C., Hartmann, D., Altevogt, P., Saftig, P. & Reiss, K. (2005). L1 is sequentially processed by two differently activated metalloproteases and presenilin/gamma-secretase and regulates neural

cell adhesion, cell migration, and neurite outgrowth. *Mol. Cell Biol.*, Vol. 25, No. 20, pp. 9040-9053.

Masaki, T., Matsuoka, H., Sugiyama, M., Abe, N., Goto, A., Sakamoto, A. & Atomi, Y. (2001). Matrilysin (MMP-7) as a significant determinant of malignant potential of early invasive colorectal carcinomas. *Br. J. Cancer*, Vol. 84, No. 10, pp. 1317-1321.

Mechtersheimer, S., Gutwein, P., gmon-Levin, N., Stoeck, A., Oleszewski, M., Riedle, S., Postina, R., Fahrenholz, F., Fogel, M., Lemmon, V. & Altevogt, P. (2001). Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. *J. Cell Biol.*, Vol. 155, No. 4, pp. 661-673.

Meng, F., Henson, R., Wehbe-Janek, H., Ghoshal, K., Jacob, S. T. & Patel, T. (2007). MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*, Vol. 133, No. 2, pp. 647-658.

Monea, S., Lehti, K., Keski-Oja, J. & Mignatti, P. (2002). Plasmin activates pro-matrix metalloproteinase-2 with a membrane-type 1 matrix metalloproteinase-dependent mechanism. *J. Cell Physiol*, Vol. 192, No. 2, pp. 160-170.

Nagase, H., Visse, R. & Murphy, G. (2006). Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc. Res.*, Vol. 69, No. 3, pp. 562-573.

Nakamura, T., Mitomi, H., Kikuchi, S., Ohtani, Y. & Sato, K. (2005). Evaluation of the usefulness of tumor budding on the prediction of metastasis to the lung and liver after curative excision of colorectal cancer. *Hepatogastroenterology*, Vol. 52, No. 65, pp. 1432-1435.

Nielsen, B. S., Jorgensen, S., Fog, J. U., Sokilde, R., Christensen, I. J., Hansen, U., Brunner, N., Baker, A., Moller, S. & Nielsen, H. J. (2011). High levels of microRNA-21 in the stroma of colorectal cancers predict short disease-free survival in stage II colon cancer patients. *Clin. Exp. Metastasis*, Vol. 28, No. 1, pp. 27-38.

Odekon, L. E., Blasi, F. & Rifkin, D. B. (1994). Requirement for receptor-bound urokinase in plasmin-dependent cellular conversion of latent TGF-beta to TGF-beta. *J. Cell Physiol*, Vol. 158, No. 3, pp. 398-407.

Okada, A., Bellocq, J. P., Rouyer, N., Chenard, M. P., Rio, M. C., Chambon, P. & Basset, P. (1995). Membrane-type matrix metalloproteinase (MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. *Proc. Natl. Acad. Sci. U. S. A*, Vol. 92, No. 7, pp. 2730-2734.

Okuyama, T., Nakamura, T. & Yamaguchi, M. (2003). Budding is useful to select high-risk patients in stage II well-differentiated or moderately differentiated colon adenocarcinoma. *Dis. Colon Rectum*, Vol. 46, No. 10, pp. 1400-1406.

Ploug, M., (2003). Structure-function relationships in the interaction between the urokinase-type plasminogen activator and its receptor. *Curr. Pharm. Des*, Vol. 9, No. 19, pp. 1499-1528.

Poulsom, R., Pignatelli, M., Stetler-Stevenson, W. G., Liotta, L. A., Wright, P. A., Jeffery, R. E., Longcroft, J. M., Rogers, L. & Stamp, G. W. (1992). Stromal expression of 72 kda type IV collagenase (MMP-2) and TIMP-2 mRNAs in colorectal neoplasia. *Am. J. Pathol.*, Vol. 141, No. 2, pp. 389-396.

Prall, F., (2007). Tumour budding in colorectal carcinoma. *Histopathology*, Vol. 50, No. 1, pp. 151-162.

Prall, F., Nizze, H. & Barten, M. (2005). Tumour budding as prognostic factor in stage I/II colorectal carcinoma. *Histopathology*, Vol. 47, No. 1, pp. 17-24.

Pyke, C., Ralfkiaer, E., Ronne, E., Hoyer-Hansen, G., Kirkeby, L. & Dano, K. (1994). Immunohistochemical detection of the receptor for urokinase plasminogen activator in human colon cancer. *Histopathology*, Vol. 24, No. 2, pp. 131-138.

Pyke, C., Romer, J., Kallunki, P., Lund, L. R., Ralfkiaer, E., Dano, K. & Tryggvason, K. (1994). The gamma 2 chain of kalinin/laminin 5 is preferentially expressed in invading malignant cells in human cancers. *Am. J. Pathol.*, Vol. 145, No. 4, pp. 782-791.

Pyke, C., Salo, S., Ralfkiaer, E., Romer, J., Dano, K. & Tryggvason, K. (1995). Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. *Cancer Res.*, Vol. 55, No. 18, pp. 4132-4139.

Remy, L., Trespeuch, C., Bachy, S., Scoazec, J. Y. & Rousselle, P. (2006). Matrilysin 1 influences colon carcinoma cell migration by cleavage of the laminin-5 beta3 chain. *Cancer Res.*, Vol. 66, No. 23, pp. 11228-11237.

Romer, J., Nielsen, B. S. & Ploug, M. (2004). The urokinase receptor as a potential target in cancer therapy. *Curr. Pharm. Des*, Vol. 10, No. 19, pp. 2359-2376.

Ronnov-Jessen, L. & Petersen, O. W. (1993). Induction of alpha-smooth muscle actin by transforming growth factor-beta 1 in quiescent human breast gland fibroblasts. Implications for myofibroblast generation in breast neoplasia. *Lab Invest*, Vol. 68, No. 6, pp. 696-707.

Roy, S., Khanna, S., Hussain, S. R., Biswas, S., Azad, A., Rink, C., Gnyawali, S., Shilo, S., Nuovo, G. J. & Sen, C. K. (2009). MicroRNA expression in response to murine myocardial infarction: miR-21 regulates fibroblast metalloprotease-2 via phosphatase and tensin homologue. *Cardiovasc. Res.*, Vol. 82, No. 1, pp. 21-29.

Sakurai, T., Lustig, M., Babiarz, J., Furley, A. J., Tait, S., Brophy, P. J., Brown, S. A., Brown, L. Y., Mason, C. A. & Grumet, M. (2001). Overlapping functions of the cell adhesion molecules Nr-CAM and L1 in cerebellar granule cell development. *J. Cell Biol.*, Vol. 154, No. 6, pp. 1259-1273.

Sato, H. & Takino, T. (2010). Coordinate action of membrane-type matrix metalloproteinase-1 (MT1-MMP) and MMP-2 enhances pericellular proteolysis and invasion. *Cancer Sci.*, Vol. 101, No. 4, pp. 843-847.

Schetter, A. J., Leung, S. Y., Sohn, J. J., Zanetti, K. A., Bowman, E. D., Yanaihara, N., Yuen, S. T., Chan, T. L., Kwong, D. L., Au, G. K., Liu, C. G., Calin, G. A., Croce, C. M. & Harris, C. C. (2008). MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. *JAMA*, Vol. 299, No. 4, pp. 425-436.

Schmid, R. S. & Maness, P. F. (2008). L1 and NCAM adhesion molecules as signaling coreceptors in neuronal migration and process outgrowth. *Curr. Opin. Neurobiol.*, Vol. 18, No. 3, pp. 245-250.

Sempere, L. F., Preis, M., Yezefski, T., Ouyang, H., Suriawinata, A. A., Silahtaroglu, A., Conejo-Garcia, J. R., Kauppinen, S., Wells, W. & Korc, M. (2010). Fluorescence-based codetection with protein markers reveals distinct cellular compartments for altered MicroRNA expression in solid tumors. *Clin. Cancer Res.*, Vol. 16, No. 16, pp. 4246-4255.

Shinto, E., Tsuda, H., Ueno, H., Hashiguchi, Y., Hase, K., Tamai, S., Mochizuki, H., Inazawa, J. & Matsubara, O. (2005). Prognostic implication of laminin-5 gamma 2 chain

expression in the invasive front of colorectal cancers, disclosed by area-specific four-point tissue microarrays. *Lab Invest*, Vol. 85, No. 2, pp. 257-266.

Sordat, I., Bosman, F. T., Dorta, G., Rousselle, P., Aberdam, D., Blum, A. L. & Sordat, B. (1998). Differential expression of laminin-5 subunits and integrin receptors in human colorectal neoplasia. *J. Pathol.*, Vol. 185, No. 1, pp. 44-52.

Stephens, R. W., Nielsen, H. J., Christensen, I. J., Thorlacius-Ussing, O., Sorensen, S., Dano, K. & Brunner, N. (1999). Plasma urokinase receptor levels in patients with colorectal cancer: relationship to prognosis. *J. Natl. Cancer Inst.*, Vol. 91, No. 10, pp. 869-874.

Strongin, A. Y., (2010). Proteolytic and non-proteolytic roles of membrane type-1 matrix metalloproteinase in malignancy. *Biochim. Biophys. Acta*, Vol. 1803, No. 1, pp. 133-141.

Suzuki, Y., Nagai, N., Umemura, K., Collen, D. & Lijnen, H. R. (2007). Stromelysin-1 (MMP-3) is critical for intracranial bleeding after t-PA treatment of stroke in mice. *J. Thromb. Haemost.*, Vol. 5, No. 8, pp. 1732-1739.

Talotta, F., Cimmino, A., Matarazzo, M. R., Casalino, L., De, V. G., D'Esposito, M., Di, L. R. & Verde, P. (2009). An autoregulatory loop mediated by miR-21 and PDCD4 controls the AP-1 activity in RAS transformation. *Oncogene*, Vol. 28, No. 1, pp. 73-84.

Tanaka, M., Hashiguchi, Y., Ueno, H., Hase, K. & Mochizuki, H. (2003). Tumor budding at the invasive margin can predict patients at high risk of recurrence after curative surgery for stage II, T3 colon cancer. *Dis. Colon Rectum*, Vol. 46, No. 8, pp. 1054-1059.

Thuault, S., Valcourt, U., Petersen, M., Manfioletti, G., Heldin, C. H. & Moustakas, A. (2006). Transforming growth factor-beta employs HMGA2 to elicit epithelial-mesenchymal transition. *J. Cell Biol.*, Vol. 174, No. 2, pp. 175-183.

Thum, T., Gross, C., Fiedler, J., Fischer, T., Kissler, S., Bussen, M., Galuppo, P., Just, S., Rottbauer, W., Frantz, S., Castoldi, M., Soutschek, J., Koteliansky, V., Rosenwald, A., Basson, M. A., Licht, J. D., Pena, J. T., Rouhanifard, S. H., Muckenthaler, M. U., Tuschl, T., Martin, G. R., Bauersachs, J. & Engelhardt, S. (2008). MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature*, Vol. 456, No. 7224, pp. 980-984.

Thurison, T., Lomholt, A. F., Rasch, M. G., Lund, I. K., Nielsen, H. J., Christensen, I. J. & Hoyer-Hansen, G. (2010). A new assay for measurement of the liberated domain I of the urokinase receptor in plasma improves the prediction of survival in colorectal cancer. *Clin. Chem.*, Vol. 56, No. 10, pp. 1636-1640.

Turner, R. R., Li, C. & Compton, C. C. (2007). Newer pathologic assessment techniques for colorectal carcinoma. *Clin. Cancer Res.*, Vol. 13, No. 22 Pt 2, pp. 6871s-6876s.

Uchiyama, S., Itoh, H., Naganuma, S., Nagaike, K., Fukushima, T., Tanaka, H., Hamasuna, R., Chijiiwa, K. & Kataoka, H. (2007). Enhanced expression of hepatocyte growth factor activator inhibitor type 2-related small peptide at the invasive front of colon cancers. *Gut*, Vol. 56, No. 2, pp. 215-226.

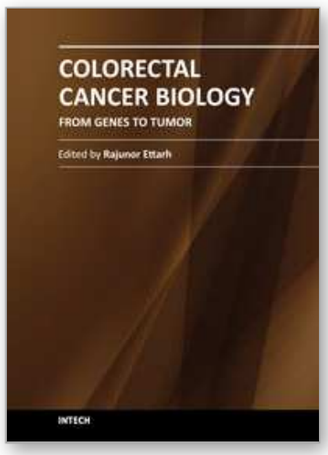
Udayakumar, T. S., Chen, M. L., Bair, E. L., Von, B., Cress, A. E., Nagle, R. B. & Bowden, G. T. (2003). Membrane type-1-matrix metalloproteinase expressed by prostate carcinoma cells cleaves human laminin-5 beta3 chain and induces cell migration. *Cancer Res.*, Vol. 63, No. 9, pp. 2292-2299.

Ueno, H., Murphy, J., Jass, J. R., Mochizuki, H. & Talbot, I. C. (2002). Tumour 'budding' as an index to estimate the potential of aggressiveness in rectal cancer. *Histopathology*, Vol. 40, No. 2, pp. 127-132.

Winter, J., Jung, S., Keller, S., Gregory, R. I. & Diederichs, S. (2009). Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell Biol.*, Vol. 11, No. 3, pp. 228-234.

Zavadil, J., Narasimhan, M., Blumenberg, M. & Schneider, R. J. (2007). Transforming growth factor-beta and microRNA:mRNA regulatory networks in epithelial plasticity. *Cells Tissues. Organs*, Vol. 185, No. 1-3, pp. 157-161.

Zlobec, I., Molinari, F., Martin, V., Mazzucchelli, L., Saletti, P., Trezzi, R., De, D. S., Vlajnic, T., Frattini, M. & Lugli, A. (2010). Tumor budding predicts response to anti-EGFR therapies in metastatic colorectal cancer patients. *World J. Gastroenterol.*, Vol. 16, No. 38, pp. 4823-4831.



Colorectal Cancer Biology - From Genes to Tumor

Edited by Dr. Rajunor Ettarh

ISBN 978-953-51-0062-1
Hard cover, 446 pages
Publisher InTech
Published online 10, February, 2012
Published in print edition February, 2012

Colorectal cancer is a common disease, affecting millions worldwide and represents a global health problem. Effective therapeutic solutions and control measures for the disease will come from the collective research efforts of clinicians and scientists worldwide. This book presents the current status of the strides being made to understand the fundamental scientific basis of colorectal cancer. It provides contributions from scientists, clinicians and investigators from 20 different countries. The four sections of this volume examine the evidence and data in relation to genes and various polymorphisms, tumor microenvironment and infections associated with colorectal cancer. An increasingly better appreciation of the complex inter-connected basic biology of colorectal cancer will translate into effective measures for management and treatment of the disease. Research scientists and investigators as well as clinicians searching for a good understanding of the disease will find this book useful.

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen