Review

Wnt up your mind – intervention strategies for S100A4-induced metastasis in colon cancer

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Abstract. Colon cancer is still a burden mainly due to metastasis formation. The latter is often associated with a constitutive activation of the Wnt/β-catenin signaling pathway and high expression of the metastasis-inducing gene S100A4. We previously identified S100A4 as a transcriptional target of β-catenin. Intervention strategies targeting Wnt/β-catenin signaling might therefore represent promising approaches to inhibit tumor growth and metastasis formation when induced by S100A4. Many inhibitors, various strategies, as well as different routes of application targeting key molecules of the Wnt signaling pathway have been reported within the last decade. Consequently, downregulation of β-catenin target genes lead to altered tumorigenic and metastastic abilities of cancer cells. This review focuses on the potential of Wnt/β-catenin signaling intervention to restrict colon cancer metastasis formation by interdicting S100A4 expression.

Keywords: Colorectal cancer — Metastasis — S100A4 — Wnt signaling — β-catenin

Abbreviations: *APC*, adenomatous polyposis coli; *CK-1α*, casein kinase-1α; *COX*, cyclooxygenase; *DKK-1*, dickkopf-1; *dn*, dominant negative; *Dvl*, dishevelled; *Fzd*, frizzled; *GSK-3β*, glycogen synthase kinase-3β; *IWP*, inhibitors of Wnt production; *LAR*, leukocyte common antigen related; *LEF-1*, lymphoid enhancer factor-1; *LRP-5/-6*, low density lipoprotein receptor-related protein-5 or -6; *MMP*, matrix metalloproteinase; *MTI*, methylation inhibitors; *NSAID*, non-steriodal anti-inflammatory drug; *PKC*, protein kinase C; *PLA2G2A*, phospholipase 2 group 2A; *PP2a*, protein phosphatase 2A; *RAGE*, receptor for advanced glycation end products; *sFRP*, secreted frizzled related protein; *tk*, transkingdom; *WIF-1*, Wnt inhibitory factor-1

Introduction

Colon carcinoma is still a major cause of cancer death worldwide. In developed countries it is the third most frequent type of cancer concerning men and second most frequent cancer occurrence in female (Garcia et al. 2007). Despite intensive health programs for early diagnosis of colon cancer, only about 40% of early stage tumors are detected. The residual 60% show already regional or even distant metastasis at the time of diagnosis.

Colon cancer in its early stages can be treated by surgery leaving patients with a good survival prognosis of about 90%. The five-year survival rate decreases to about 65% when patients are diagnosed with lymph node metastasis. However, a drastic reduction of the five-year survival rate to about 10% is observed when distant metastases have formed. In conclusion, approximately 90% of all colon cancer deaths arise from metastasis dissemination of primary tumors (Stein and Schlag 2007).

Efforts to identify main molecular players in metastasis formation revealed the metastasis progressor protein S100A4 (Ebralidze et al. 1989). We demonstrated that S100A4 was useful as a prognostic biomarker for metachronous colon cancer metastasis formation. Thus, S100A4 contributes to the early identification of patients with high risk to develop distant metastasis. Moreover, we also identified S100A4 as one of the target genes of the canonical Wnt/β-catenin pathway (Stein et al. 2006). Furthermore, S100A4 was found to be

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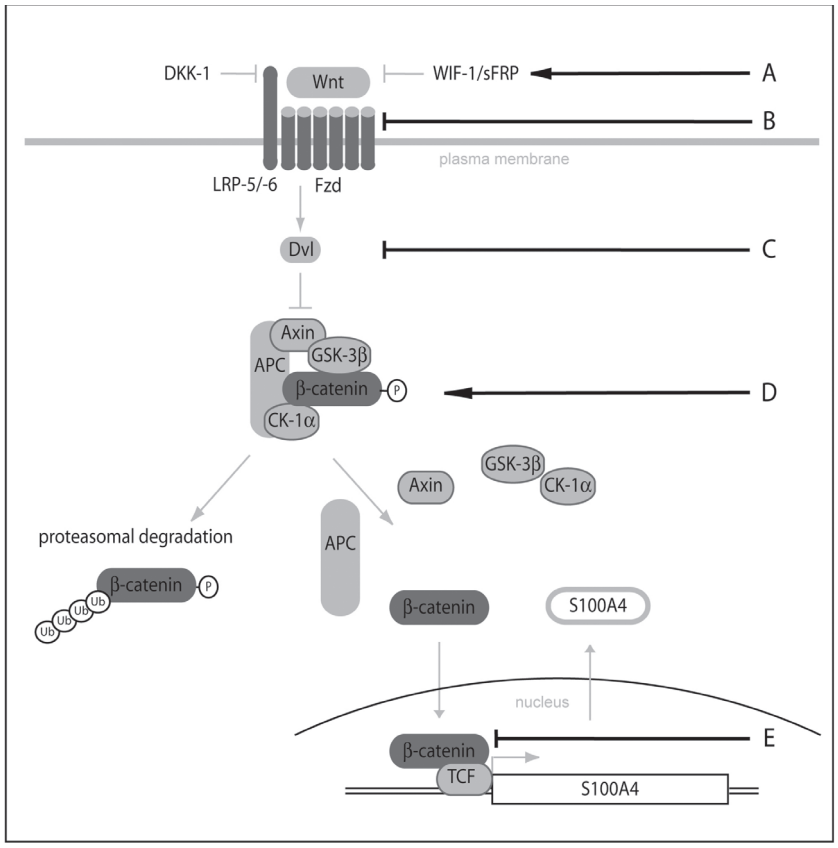

Figure 1. Potential target sites within the canonical Wnt/β-catenin signaling pathway for inhibition of S100A4 induced metastasis formation (adapted from Barker and Clevers 2006; Barker 2008; Klaus and Birchmeier 2008).
A. Activation of naturally occurring Wnt antagonists: DKK-1, WIF-1, sFRP.
B. Inhibition of Wnt receptor signaling initiation complex: Wnt; Fzd, LRP-5/-6.
C. Inhibition of Wnt signal transduction *via* inhibiting Dvl.
D. Re-activation of the β-catenin destruction complex: APC, Axin; GSK-3β, CK-1α.
E. Prevention/Disruption of the β-catenin/TCF transcription activating complex.

specifically expressed at the invasion front of colorectal cancer together with nuclear β-catenin (Hlubek et al. 2007). The link of S100A4 expression to the Wnt/β-catenin pathway was also shown for gastric cancer (Yoon et al. 2008; Li et al. 2008). In osteosarcoma, metastasis formation was also mediated by the regulation of S100A4 through the Wnt signaling pathway (Guo et al. 2009). Many inhibitors and intervention strategies targeting the Wnt signaling pathway have been reported within the last decade. This review focuses on their potential to restrict S100A4 induced metastasis formation (Fig. 1).

I. Wnt signaling in metastasis formation of colorectal cancer

Active Wnt signaling is crucial for cell differentiation along the crypt-villus axis and hence plays a central role in maintaining the homeostasis of colon tissue (Pinto et al. 2003). Consequently, deregulation of this important signaling pathway is a major event in tumorigenesis of colon carcinoma (Segditsas and Tomlinson 2006). Although further pathways such as K-ras, Notch/bHLH, BMP-4 and hedgehog signaling contribute to colorectal cancer, the Wnt signaling pathway plays a decisive role for this cancer entity (Klaus and Birchmeier 2008; Fre et al. 2008).

The Wnt signaling pathway can be distinguished between the non-canonical and the canonical signaling pathway. The non-canonical Wnt signaling pathway regulates cell adhesion, cell polarity and coordinated tissue movement (Vincan and Barker 2008) *via* binding of β-catenin to the cytoplasmic region of cadherin adhesion receptors (Jeanes et al. 2008). The canonical Wnt signaling pathway regulates cell differentiation, proliferation, migration and invasion by strictly controlling the amount of cytoplasmic β-catenin (Barker

2008). In the absence of active Wnt signaling, β-catenin levels are reduced by the destruction complex. In this complex β-catenin is anchored by APC and Axin which builds a platform for the protein kinases CK-1α and GSK-3β and the protein phosphatase 2A (PP2A) to enter the complex and sequentially phosphorylate β-catenin (Komiya and Habas 2008). Phosphorylation of β-catenin generates a binding site for E3 ubiquitin ligase, which leads to polyubiquitinylation and rapid proteasomal degradation. Suppressed levels of β-catenin enable Groucho proteins to interact with T-cell factor (TCF)/lymphoid enhancer factor-1 (LEF-1) transcription factors and Wnt signaling enhancers to inhibit transcription of β-catenin target genes (Behrens et al. 1996; Brabletz et al. 1998; Barker 2008).

Activation of the Wnt/β-catenin signaling pathway is initiated by binding of secreted Wnt proteins to their receptor from the Frizzled (Fzd) family and their co-receptors LRP-5/-6 at the plasma membrane. The formed Wnt-Fzd-LRP-5/-6 initiation complex triggers the association of dishevelled (Dvl) with the cytoplasmic tail of Fzd receptor. That activates phosphorylation of LRP-5/-6 and further sequesters Axin from the destruction complex to the plasma membrane. Disruption of the destruction complex leads to accumulation of cytoplasmic β-catenin which then translocates into the nucleus (Barker 2008). Nuclear β-catenin displaces Groucho proteins from their complex with TCF/LEF-1 transcription factors and initiates transcription of β-catenin target genes (Daniels and Weis 2005). This target gene transcription is further amplified by the recruitment of transcriptional enhancers such as CBT, TBP, BRG-1, legless, Bcl-9, mediator or hyrax (Barker 2008).

In non-cancer cells Wnt signaling is tightly controlled by its antagonists. They can be divided into two functional classes: the secreted frizzled related protein (sFRP) class and the dickkopf (DKK) class. Proteins of the sFRP class such as sFRP family members, Wnt inhibitor factor-1 (WIF-1) and Cerberus bind directly to secreted Wnts and thereby diminish non-canonical and canonical Wnt signaling. In contrast, proteins of the DKK class specifically inhibit canonical Wnt signaling by binding to LRP-5/-6 and interfering with the formation of the Wnt receptor signaling complex (Kawano and Kypta 2003).

In a major part of colon cancers the canonical Wnt/β-catenin pathway is constitutively active. About 90% of colorectal cancers bear mutations in the APC gene, which lead to a disruption of the β-catenin destruction complex and consequently to a nuclear accumulation of β-catenin. Another set of colorectal cancers displays a loss of conserved β-catenin phosphorylation sites which results in abolished proteasomal degradation. Constitutive Wnt signaling can further be potentiated by deregulated and thus overexpressed proteins of the Wnt and Fzd family (Vincan and Barker 2008). Deregulation of Wnt signaling in colon cancer leads to aggressive tumor growth and poor prognosis. One of the Wnt target genes that directly promotes this increased metastatic potential is S100A4 (Stein et al. 2006).

II. S100A4 promotes metastasis formation in many types of cancer

S100A4 was firstly discovered in 1989 by Ebralizde *et al.* who found it overexpressed in metastatic cells (Ebralidze et al. 1989). S100A4 is a 11 kDa small Ca^{2+}-binding protein which is encoded in the epidermal differentiation complex located on human chromosome 1 (1q21). This region is frequently rearranged in human cancers (Fernandez-Fernandez et al. 2008). Consistently, S100A4 is overexpressed in many different types of cancer such as gallbladder, bladder, breast, oesophageal, gastric, pancreatic, hepatocellular, non-small lung, and colorectal cancer, and its presence highly correlates with the aggressiveness of a tumor (Helfman et al. 2005). Furthermore, it was found that S100A4 expression levels positively correlate with the chance of a tumor to metastasize. S100A4 itself is not necessarily tumorigenic since transgenic mice overexpressing S100A4 do not develop tumors *per se* (Ambartsumian et al. 1996). However, when crossed with MMTV-neu or GRS/A mice which are characterized by forming mammary tumors that rarely metastasize, S100A4 overexpression leads to highly aggressive primary tumors and formation of metastasis (Davies et al. 1996; Ambartsumian et al. 1996). On the other hand, S100A4 null mice injected with highly metastatic mouse mammary carcinoma cells displayed no metastases (Grum-Schwensen et al. 2005). These observations suggest that S100A4 is not simply a marker for metastatic disease but rather has a causal role in mediating this process. Large efforts have been made in the last years to investigate the mechanisms by which S100A4 promotes metastasis formation.

S100A4 protein activity is dependent on intracellular Ca^{2+}

S100A4 is one of the currently known 25 members of the S100 protein family that share high sequential and structural homology. Their structure consists of two EF hands connected by a hinge region (Gingras et al. 2008). EF hands are defined by a helix-loop-helix motif and the loop creates a binding pocket for one Ca^{2+}-ion. Additionally to the canonical EF hand at the C-terminus S100 proteins are characterized by their N-terminal pseudo EF hand that comprises a slightly elongated loop region. This elongation results in a lower affinity to Ca^{2+} which allows ion binding to occur sequentially (Dutta et al. 2002). Ca^{2+}-binding triggers a conformational shift which generates two major hydrophobic binding sites partly formed by the hinge region (Pathuri et al. 2008). Residues of this hinge region as well as the lengthy C-terminal tail

of S100A4 share almost no sequence homology within the S100 protein family. Hence those regions confer specificity towards S100A4 protein interaction partners (Vallely et al. 2002). Several protein binding partners were identified, such as proteins of the cytoskeleton, the tumor suppressor protein p53, the receptor for advanced glycation end products (RAGE), and annexin II.

Intracellular S100A4 increases cell motility

S100A4 directly increases cell motility by its interaction with proteins of the cytoskeleton. It was found to coaggregate with actin filaments in sedimentation assays (Watanabe et al. 1993) and to bind non-muscle tropomyosin (Takenaga et al. 1994). However, the most prominent binding partner of S100A4 is non-muscle myosin II (Chen et al. 2001). Myosin II is a chemomechanical protein that participates in cell division, cell motility and secretion. S100A4 binding to myosin II inhibits myosin polymerization and even promotes the disassembly of myosin filaments at the leading edge of migrating cells. This local enrichment of myosin II monomers is needed for the formation of flexible directed protrusions as a first step in migration (Li et al. 2003; Li and Bresnick 2006).

Besides increasing cell motility and cell polarization S100A4 affects cell adhesion by binding to liprin β1. Liprin β1 itself interacts with a transmembrane protein tyrosine phosphatase called leukocyte common antigen related (LAR) which is localized at the end of focal adhesions. It is suggested that S100A4 affects LAR function by binding to liprin β1 and thus modulates cell adhesion causing a migratory phenotype (Kriajevska et al. 2002).

Grigorian *et al.* firstly discovered the binding of S100A4 to the tumor suppressor p53 and the modulation of its transcriptional activity. Additionally, binding of S100A4 masks several C-terminal PKC phosphorylation sites of p53 (Grigorian et al. 2001). Although the meaning of the phosphorylation status of p53 is still controversially debated, inhibition of PKC phosphorylation can stabilize p53 (Chernov et al. 1998). Furthermore, increased p53 levels lead to increased migration and invasion rates in colon cancer cell lines (Sablina et al. 2003). However, the exact role of S100A4 in p53 mediated migration still needs to be further elucidated.

Extracellular S100A4 induces metastasis formation and angiogenesis

Beside its intracellular interactions, S100A4 is released to the extracellular space and initiates metastasis formation and angiogenesis. For instance, exogenously added oligomeric S100A4 increased cell motility of endothelial cells *in vitro* (Ambartsumian et al. 2001). Injection of recombinant S100A4 protein increased metastasis formation of a mouse mammary adenocarcinoma cell line *in vivo* when tumor cells were in contact with stroma cells. This also implies a role for S100A4 to enhance intercellular communication. S100A4, when secreted by stroma cells, further increased vascularization of the tumor (Schmidt-Hansen et al. 2004a). Extracellular S100A4 activates expression of matrix metalloproteinases (MMPs) such as MMP-1, -3, -9 and 13 (Schmidt-Hansen et al. 2004b; Senolt et al. 2006). MMPs cleave proteins of the extracellular matrix and thereby enable cell invasion into adjacent tissues. In chondrocytes S100A4 induces MMP-13 expression via binding to RAGE (Yammani et al. 2006). However, extracellular S100A4 can also activate expression of MMP-13 independently of RAGE via NF-κB (Schmidt-Hansen et al. 2004b).

Furthermore, S100A4 also promotes angiogenesis. Extracellular S100A4 dimers form heterotetramers with annexin II dimers. This activates the plasminogen activated system which stimulates MMPs and together facilitates angiogenesis (Semov et al. 2005).

III. Targeting the Wnt/β-catenin pathway to suppress S100A4 expression

Concentrating on the regulation of S100A4 expression, we identified S100A4 as a target gene of the canonical Wnt/β-catenin pathway. We compared gene expression profiles of the human colon carcinoma cell line HCT116, which carries one allele with a gain-of-function mutation of β-catenin and a wildtype β-catenin allele, with the derivative cell line HAB-92^{wt}, where the mutant β-catenin allele was ablated by homologous recombination (Kim et al. 2002). In this experiment we found a massive upregulation of S100A4 in cells with mutant β-catenin due to an increased level of nuclear β-catenin. The promoter analysis of S100A4 showed a direct regulation by the β-catenin/TCF complex. Effects on both cell migration and invasion induced by constitutive nuclear β-catenin are mediated by S100A4 and were significantly reduced upon S100A4 siRNA application. The identification of S100A4 as a target gene of the canonical Wnt/β-catenin pathway provides the link between two previously unconnected molecular pathways which play important roles in tumor progression and metastasis formation in colorectal cancer (Stein et al. 2006). This finding constitutes a new basis for improved anti-metastatic treatment in colon cancer since the Wnt/β-catenin pathway contains several potential sites to interfere with active signaling.

Blockage of the Wnt-Fzd-LRP-5/-6 initiation complex

The effect of active β-catenin to transform a tumor towards a more aggressive state by activating S100A4 can be blocked by interfering with the interaction of Wnt and its receptors. Successful inhibition of Wnt-1 signaling was achieved by

application of siRNA in human cancer cells *in vitro*. Treatment of Wnt-1 overexpressing cell lines with Wnt-1 siRNA resulted in increased apoptosis. *In vivo* tumor growth was inhibited by application of a specific Wnt-1 antibody (He et al. 2004). Wnt-2b is a mediator of the mesenchymal-epithelial transition needed for secondary tumor formation. Treatment of xenograft models with a specific Wnt-2b antibody also resulted in decreased tumor growth (You et al. 2004). These data present the possibility to abort the interaction of secreted Wnt proteins with their Fzd receptors in a highly specific manner. Therefore, it is promising to investigate whether targeting other proteins of the Wnt family could prevent S100A4 induced migration despite of inducing apoptosis.

Besides targeting the interaction between Wnt and Fzd receptors, inhibiting secretion of Wnts interferes at the very beginning of Wnt signaling. Chen *et al.* recently reported on the identification of a group of small molecules they defined as inhibitors of Wnt production (IWP). Those IWPs all blocked porcupine, an acetyltransferase needed for palmitoylation and thus the secretion of Wnts. Interestingly, the blockage of Wnt secretion was effective to abort Wnt signaling despite the presence of mutated APC and thus constitutive activity of the pathway (Chen et al. 2009). The ability of those inhibitors to prevent S100A4 expression in human colon cancer and thus S100A4 induced metastasis would be a worthwhile investigation.

Disruption of the Wnt-Fzd-LRP-5/-6 signaling initiation complex can also occur by targeting the co-receptors LRP-5/-6. For instance, knockdown of LRP-5 expression by siRNA in human mesenchymal stem cells resulted in a decreased ability of the cells to invade (Neth et al. 2006). Colony formation, cell migration and invasion rates of osteosarcoma cells were reduced when dominant negative LRP-5 (dnLRP-5) was stably expressed. DnLRP-5 competes with endogenous LRP-5 in the Wnt signaling initiation complex and thus disrupts Wnt signaling. *In vivo* dnLRP-5 expressing cells reduced the number and size of lung metastasis in a spontaneous pulmonary metastasis model (Guo et al. 2008). Therefore, dnLRP-5 treatment also bears great potential to be applied against S100A4 induced metastasis.

Abortion of Wnt-Fzd downstream signaling

Wnt signaling is initiated by formation of the Wnts-Fzd-LRP-5/6 receptor complex. Intracelullar downstream transmission of the signal is dependent on Dvl proteins. Upon Wnt signaling Dvl activates recruitment of Axin to the plasma membrane and thereby disrupts the β-catenin destruction complex. Dvl proteins are overexpressed in many cancers leading to constitutive β-catenin signaling (Mizutani et al. 2005).

Dvl proteins consist of three functional domains of which the central PDZ domain is essential for protein-protein interaction. Via this domain Dvl binds to the intracellular part of Fzd receptors. This binding is essential for transmission of Wnt signals. Accordingly, deletion of the PDZ domain in Dvl protein resulted in a drastic reduction of β-catenin target gene expression (Uematsu et al. 2003b). Since the structure of the PDZ domain of Dvl is known (Khlebtsova et al. 2000), Shan *et al.* applied computational structure-based ligand screening to identify potential Dvl inhibitors (Shan et al. 2005). Virtual 3D screening revealed the small molecule NSC668036 which was validated to bind to the PDZ domain of Dvl and further to inhibit Wnt-3a induced secondary axis formation.

By creating a chemical library which was applied in a high throughput screening, You *et al.* identified a new small molecule called FJ9. FJ9 inhibited Dvl induced Wnt activation in HEK293 cells which resulted in decreased TCF-dependent transcription. Furthermore, FJ9 suppressed tumor growth *in vivo* (You et al. 2008). By large scale screening of peptides that can bind to the PDZ domain of Dvl-2, Zhang *et al.* recently published the peptide pen-N3 to inhibit Wnt signaling. Pen-N3 inhibited Dvl induced β-catenin/TCF reporter signaling to comparable extents as FJ9. Pen-N3 was nontoxic at Wnt inhibiting concentrations (Zhang et al. 2009).

Targeting Dvl protein with siRNA resulted in growth inhibition and reduced colony formation in lung cancer cells. However, in colon cancer cells that bear mutated APC Dvl siRNA had no effect on cellular growth (Uematsu et al. 2003a).

Those promising data on blocking Wnt signaling by acting on Dvl offer alternative strategies to reduce S100A4 expression and might thus be applied in anti-metastatic treatment.

Stimulation of the destruction complex

Stabilization or reactivation of the β-catenin destruction complex bears great potential to restrict constitutive Wnt/β-catenin signaling. Calcimycin is a Ca^{2+}-ionophor that increases intracellular Ca^{2+} by binding extracellular Ca^{2+} and shuffling it into the cell. Calcimycin was found to stimulate β-catenin destruction by activating protein kinase C (PKC). Upon increased intracellular Ca^{2+}-levels, PKC phosphorylates β-catenin and stimulates proteasomal degradation of the latter (Gwak et al. 2006). Calcimycin treatment of hematopoetic cells resulted in a decrease of S100A4 mRNA level (Grigorian et al. 1994). Combining those two findings, calcimycin could be applied against S100A4 induced metastasis.

Another strategy to stimulate the β-catenin destruction complex is the treatment with dexamethasone. In osteoblasts dexamethasone stimulated the upregulation of Axin-2 which

is the scaffold protein of the destruction complex (Hayashi et al. 2009). Moreover, induced Axin stabilization in colorectal cancer cells was recently reported. A panel of small molecules was able to block accumulation of free β-catenin that was not in complex with E-cadherin by elevating Axin levels in the cell. Strikingly, increased Axin levels compensated the loss of APC tumor suppressor function (Chen et al. 2009). The potential of those compounds to reduce S100A4 induced metastasis formation is a promising approach for future research.

RNA interference technology can also be applied to target pathway key molecules such as β-catenin. Very recently, an interesting *in vivo* approach using transkingdom(tk)RNAi against β-catenin was reported. The application of tkRNAi led to reduced β-catenin expression in the mucosa, when delivered to the gastrointestinal tract after oral feeding (Silva et al. 2009).

Prevention/Disruption of the β-catenin/TCF complex

Non-steriodal anti-inflammatory drugs (NSAIDs) have been repeatedly evaluated as potential Wnt/β-catenin pathway therapeutics. Prominent Wnt signaling inhibition by NSAIDs was recently investigated in clinical studies (e.g. Meyskens et al. 2008; Sporn and Hong 2008; Tuma 2008). Traditional NSAIDs include aspirin, sulindac or indomethacin that all inhibit cyclooxygenase (COX) activation. Elevated COX expression levels in cancer lead to increased prostaglandin levels which subsequently activate Wnt signaling. Therefore reduction of COX signaling via NSAIDs can reduce Wnt-signaling and induce β-catenin degradation (Tuynman et al. 2008).

For instance, sulindac has been well established as a colon cancer chemopreventive agent for many years. It has pleiotropic activities as a COX inhibitor and as an inhibitor of polyamine biosynthesis. However, it is less known, that sulindac also acts *via* its inhibition of the nuclear accumulation and expression of β-catenin. Sulindac inhibits the nuclear accumulation of β-catenin in colon carcinoma cell lines, but also in adenomas of patients with familial adenomatous polyposis leading to reduced downstream signaling (Orner et al. 2003; Rice et al. 2003; Boon et al. 2004). Moreover, sulindac inhibits β-catenin expression in colorectal cancer cells and also in patients with hereditary nonpolyposis colorectal cancer and familial adenomatous polyposis (Gardner et al. 2004; Koornstra et al. 2005; Han et al. 2008). As a consequence, upregulated target genes of β-catenin, like Met and cyclin D1, are downregulated following sulindac treatment (Boon et al. 2004; Dihlmann and von Knebel Doeberitz 2005).

For other molecules such as silibinin, a flavonolignan extracted from milk thisthle (*Silybum marianum*) plant, the modulation of the Wnt/β-catenin cascade has been reported. The caused decrease of β-catenin expression resulted in downregulation of target genes such as cyclin D1 and c-myc and inhibited the spontaneous intestinal tumorigenesis in $APC^{min/+}$ mice (Rajamanickam et al. 2009).

Since the artificial disruption of the β-catenin/TCF complex consequently results in target gene inactivation, many efforts have been made to identify small molecule inhibitors that are able to disrupt the β-catenin/TCF interaction. By high throughput screening, natural compounds were found, e.g. PKF115-584, PKF222-815, and CPG049090, that target the binding of β-catenin to TCF. Their cancer therapeutic potential has been shown by blocking growth of colon cancer cell lines (Lepourcelet et al. 2004; Barker and Clevers 2006).

Thus, inhibitors of the β-catenin/TCF complex may also offer potential as anti-metastatic agents by interdicting S100A4 expression. It might serve as the basis for chemoprevention of metastasis formation in individual colon cancer patients at high metastatic risk.

Activation of naturally occurring Wnt antagonists

Wnt antagonist expression is repressed in many types of cancer mainly due to promoter hypermethylation. Thereby, constitutively active Wnt signaling is provoked. For instance, the DKK-1 promoter was found to be hypermethylated in colon cancer (Aguilera et al. 2006). Furthermore, WIF-1 promoter hypermethylation frequently occurs in gastrointestinal tumors resulting in loss of WIF-1 expression and its negative regulation of the Wnt/β-catenin pathway (Taniguchi et al. 2005). Decreased expression of phospholipase 2G2A (PLA2G2A) was also found in late tumor stages of gastric cancer. PLA2G2A is a direct target of the Wnt/β-catenin signaling pathway which was recently identified as Wnt antagonist. Its expression correlated with the inhibition of S100A4 expression and the reduced ability of a tumor to metastasize (Ganesan et al. 2008).

Transcriptional reactivation of Wnt antagonist expression showed promising anti-cancerous results *in vitro*. For transcriptional reactivation methylation inhibitors (MTI) are useful drugs and many of them have already shown promising results in clinical trials. However, MTIs act on DNA methyltransferases and thus mainly activate transcription in general. To act more specific on the Wnt/β-catenin pathway, overexpression of naturally occurring Wnt antagonist represent an alternative opportunity to reduce β-catenin target gene expression and thereby S100A4 induced metastasis formation.

Conclusion and future perspectives

Colon cancer is still an unsolved burden mainly due to metastasis formation. Therefore, successful therapies need to target right at these cellular and molecular processes.

The Wnt/β-catenin pathway is constitutively active in almost all colon cancers which leads to highly metastasizing phenotypes. S100A4 as a target gene of this pathway is one of the main promoters of metastasis formation. Strikingly, S100A4 was shown to function as a precious biomarker for the identification of patients with high risk to develop metastases metachronously or who already have developed metastases. For those patients the demonstrated intervention strategies provide high potential to improve their survival prognosis.

Many approaches were made to identify S100A4 inhibitors that target the interactions of S100A4 with its target proteins. However, the role of S100A4 in metastasis formation is manifold. It acts on cellular adhesion to further increase cell motility. Furthermore, secreted S100A4 initiates cell invasion and angiogenesis. With all those various actions it still needs to be shown that specific inhibition of S100A4 protein is sufficient to restrict metastasis formation. Targeting the Wnt/β-catenin pathway to inhibit S100A4 gene expression bears the huge advantage to generally inhibit all S100A4 functions at once.

We certainly know of the complexity of this pathway, of the various described β-catenin target genes, as well as of the lack of strict specificity of the compounds mentioned here. The specificity of each Wnt signaling pathway-based intervention approach towards downregulation of S100A4 expression should be evaluated to prevent unwanted effects in the tumors as well as in the non-tumor tissues. Thus, the evaluation of potential side effects should be balanced together with the therapeutic potential with respect to metastasis reduction in preclinical studies.

Moreover, targeting exclusively the Wnt/β-catenin pathway can be insufficient in some cases, since other signaling cascades can also impinge on the expression of S100A4. Beside Wnt signaling, for instance, ErbB2 signals can activate S100A4 expression in medulloblastoma cell lines during EMT via the Ras/Raf/Mek/Erk1/2 signaling pathways (Hernan et al. 2003). Additionally, more recent studies by O'Connor and colleagues identified α6β4 integrin to activate S100A4 expression in breast cancer cell lines *via* NFAT5 transcription factor (Chen et al. 2009).

Each pathway might be active to a different extent in distinct types of tumors, and should be therefore differentially used as therapeutic target. The role of each transcriptional regulation in colon cancer needs to be further evaluated, before specific intervention strategies can be designed. The decisive impact of the Wnt/β-catenin signaling pathway, however, is without any doubt. A major aim of future therapies is the S100A4-based early identification of high risk patients for metastasis intervention strategies. Targeting the Wnt signaling pathway offers promising approaches for the ultimate goal – the reduction or even prevention of the S100A4-induced colon cancer metastasis.

Acknowledgement. We grateful thank Wolfgang Walther, Dennis Kobelt and Mathias Dahlmann for their critical reading and helpful discussions.
The authors have no financial interests related to the material in the manuscript nor to the participation in the 2nd ECS Workshop.

References

Aguilera O., Fraga M. F., Ballesta E., Paz M. F., Herranz M., Espada J., García J. M. Muñoz A., Esteller M., González-Sancho J. M. (2006): Epigenetic inactivation of the Wnt antagonist DICKKOPF-1 (DKK-1) gene in human colorectal cancer. Oncogene **25,** 4116–4121

Ambartsumian N. S., Grigorian M. S., Larsen I. F., Karlstrøm O., Sidenius N., Rygaard J., Georgiev G., Lukanidin E. (1996): Metastasis of mammary carcinomas in GRS/A hybrid mice transgenic for the mts1 gene. Oncogene **13,** 1621–1630

Ambartsumian N., Klingelhöfer J., Grigorian M., Christensen C., Kriajevska M., Tulchinsky E., Georgiev G., Berezin V., Bock E., Rygaard J., Cao R., Cao Y., Lukanidin E. (2001): The metastasis-associated Mts1(S100A4) protein could act as an angiogenic factor. Oncogene **20,** 4685–4695

Barker N. (2008): The canonical Wnt/beta-catenin signalling pathway. Methods Mol. Biol. **468,** 5–15

Barker N., Clevers H. (2006): Mining the Wnt pathway for cancer therapeutics. Nat. Rev. Drug Discov. **5,** 997–1014

Behrens J., von Kries J. P., Kühl M., Bruhn L., Wedlich D., Grosschedl R., Birchmeier W. (1996): Functional interaction of beta-catenin with the transcription factor LEF-1. Nature **382,** 638–642

Boon E. M., Keller J. J., Wormhoudt T. A., Giardiello F. M., Offerhaus G. J., van der Neut R., Pals S. T. (2004): Sulindac targets nuclear beta-catenin accumulation and Wnt signalling in adenomas of patients with familial adenomatous polyposis and in human colorectal cancer cell lines. Br. J. Cancer **90,** 224–229

Brabletz T., Jung A., Hermann K., Günther K., Hohenberger W., Kirchner T. (1998): Nuclear overexpression of the oncoprotein beta-catenin in colorectal cancer is localized predominantly at the invasion front. Pathol. Res. Pract. **194,** 701–714

Chen B., Dodge M. E., Tang W., Lu J., Ma Z., Fan C., Wei S., Hao W., Kilgore J., Williams N. S., Roth M. G., Amatruda J. F., Chen C., Lum L. (2009): Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat. Chem. Biol. **5,** 100–107

Chen H., Fernig D. G., Rudland P. S., Sparks A., Wilkinson M. C., Barraclough R. (2001): Binding to intracellular targets of the metastasis-inducing protein, S100A4 (p9Ka). Biochem. Biophys. Res. Commun. **286,** 1212–1217

Chen M., Sinha M., Luxon B. A., Bresnick A. R., O'Connor K. L. (2009): Integrin α6β4 controls the expression of genes associated with cell motility, invasion, and metastasis, including S100A4/metastasin. J. Biol. Chem. **284,** 1484–1494

Chernov M. V., Ramana C. V., Adler V. V., Stark G. R. (1998): Stabilization and activation of p53 are regulated independently by different phosphorylation events. Proc. Natl. Acad. Sci. U.S.A. **95,** 2284–2289

Daniels D. L., Weis W. I. (2005): β-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. Nat. Struct. Mol. Biol. **12,** 364–371

Davies M. P., Rudland P. S., Robertson L., Parry E. W., Jolicoeur P., Barraclough R. (1996): Expression of the calcium-binding protein S100A4 (p9Ka) in MMT-Vneu transgenic mice induces metastasis of mammary tumours. Oncogene **13,** 1631–1637

Dihlmann S., von Knebel Doeberitz M. (2005): Wnt/beta-catenin-pathway as a molecular target for future anti-cancer therapeutics. Int. J. Cancer **113,** 515–524

Dutta K., Cox C. J., Huang H., Basavappa R., Pascal S. M. (2002): Calcium coordination studies of the metastatic Mts1 protein. Biochemistry **41,** 4239–4245

Ebralidze A., Tulchinsky E., Grigorian M., Afanasyeva A., Senin V., Revazova E., Lukanidin E. (1989): Isolation and characterization of a gene specifically expressed in different metastatic cells and whose deduced gene product has a high degree of homology to a Ca^{2+}-binding protein family. Genes Dev. **3,** 1086–1093

Fernandez-Fernandez M. R., Rutherford T. J., Fersht A. R. (2008): Members of the S100 family bind p53 in two distinct ways. Protein Sci. **17,** 1663–1670

Fre S., Vignjevic D., Schoumacher M., Duffy S. L., Janssen K., Robine S., Louvard D. (2008): Epithelial morphogenesis and intestinal cancer: new insights in signaling mechanisms. Adv. Cancer Res. **100,** 85–111

Ganesan K., Ivanova T., Wu Y., Rajasegaran V., Wu J., Lee M. H., Yu K., Rha S. Y., Chung H. C., Ylstra B., Meijer G., Lian K. O., Grabsch H., Tan P. (2008): Inhibition of gastric cancer invasion and metastasis by PLA2G2A, a novel beta-catenin/TCF target gene. Cancer Res. **68,** 4277–4286

Garcia M., Jemal A., Ward E. M., Center M. M., Hao Y., Siegel R. L., Thun M. J. (2007): Global Cancer Facts & Figures 2007. Atlanta, GA: American Cancer Society

Gardner S. H., Hawcroft G., Hull M. A. (2004): Effect of nonsteroidal anti-inflammatory drugs on beta-catenin protein levels and catenin-related transcription in human colorectal cancer cells. Br. J. Cancer **91,** 153–163

Gingras A. R., Basran J., Prescott A., Kriajevska M., Bagshaw C. R., Barsukov I. L. (2008): Crystal structure of the Ca^{2+}-form and Ca^{2+}-binding kinetics of metastasis-associated protein, S100A4. FEBS Lett. **582,** 1651–1656

Grigorian M., Andresen S., Tulchinsky E., Kriajevska M., Carlberg C., Kruse C., Cohn M., Ambartsumian N., Christensen A., Selivanova G., Lukanidin E. (2001): Tumor suppressor p53 protein is a new target for the metastasis-associated Mts1/S100A4 protein. J. Biol. Chem. **276,** 22699–22708

Grigorian M., Tulchinsky E., Burrone O., Tarabykina S., Georgiev G., Lukanidin E. (1994): Modulation of mts1 expression in mouse and human normal and tumor cells. Electrophoresis **15,** 463–468

Grum-Schwensen B., Klingelhofer J., Berg C. H., El-Naaman C., Grigorian M., Lukanidin E., Ambartsumian N. (2005): Suppression of tumor development and metastasis formation in mice lacking the S100A4(mts1) Gene. Cancer Res. **65,** 3772–3780

Guo Y., Rubin E., Xie J., Zi X., Hoang B. (2008): Dominant negative LRP5 decreases tumorigenicity and metastasis of osteosarcoma in an animal model. Clin. Orthop. Relat. Res. **466,** 2039–2045

Guo Y., Xie J., Zi X., Hoang B. (2009): Wnt signaling regulates S100A4 to mediate metasis of osteosarcoma. Proc. Am. Assoc. Cancer Res. **50,** 531–532

Gwak J., Cho M., Gong S., Won J., Kim D., Kim E., Lee S. S., Kim M., Kim T. K., Shin J., Oh S. (2006): Protein-kinase-C-mediated β-catenin phosphorylation negatively regulates the Wnt/β-catenin pathway. J. Cell Sci. **119,** 4702–4709

Han A., Song Z., Tong C., Hu D., Bi X., Augenlicht L. H., Yang W. (2008): Sulindac suppresses beta-catenin expression in human cancer cells. Eur. J. Pharmacol. **583,** 26–31

Hayashi K., Yamaguchi T., Yano S., Kanazawa I., Yamauchi M., Yamamoto M., Sugimoto T. (2009): BMP/Wnt antagonists are upregulated by dexamethasone in osteoblasts and reversed by alendronate and PTH: Potential therapeutic targets for glucocorticoid-induced osteoporosis. Biochem. Biophys. Res. Commun. **379,** 261–266

He B., You L., Uematsu K., Xu Z., Lee A. Y., Matsangou M., McCormick F., Jablons D. M. (2004): A monoclonal antibody against Wnt-1 induces apoptosis in human cancer cells. Neoplasia **6,** 7–14

Helfman D. M., Kim E. J., Lukanidin E., Grigorian M. (2005): The metastasis associated protein S100A4: role in tumour progression and metastasis. Br. J. Cancer **92,** 1955–1958

Hernan R., Fasheh R., Calabrese C., Frank A. J., Maclean K. H., Allard D., Barraclough R., Gilbertson R. J. (2003): ERBB2 up-regulates S100A4 and several other prometastatic genes in medulloblastoma. Cancer Res. **63,** 140–148

Hlubek F., Brabletz T., Budczies J., Pfeiffer S., Jung A., Kirchner T. (2007): Heterogeneous expression of Wnt/beta-catenin target genes within colorectal cancer. Int. J. Cancer **121,**1941–1948

Jeanes A., Gottardi C. J., Yap A. S. (2008): Cadherins and cancer: how does cadherin dysfunction promote tumor progression? Oncogene **27,** 6920-6929

Kawano Y., Kypta R. (2003): Secreted antagonists of the Wnt signalling pathway. J. Cell Sci. **116,** 2627–2634

Khlebtsova N., Hung L. W., Henderson K., Moon R., Earnest T. (2000): Expression, crystallization and preliminary X-ray studies of the PDZ domain of Dishevelled protein. Acta Crystallogr. D Biol. Crystallogr. **56,** 212–214

Kim J. S., Crooks H., Dracheva T., Nishanian T. G., Singh B., Jen J., Waldman T. (2002): Oncogenic beta-catenin is required for bone morphogenetic protein 4 expression in human cancer cells. Cancer Res. **62,** 2744–2748

Klaus A., Birchmeier W. (2008): Wnt signalling and its impact on development and cancer. Nat. Rev. Cancer **8**, 387–398

Komiya Y., Habas R. (2008): Wnt signal transduction pathways. Organogenesis **4,** 68–65

Koornstra J. J., Rijcken F. E., Oldenhuis C. N., Zwart N., van der Sluis T., Hollema H., deVries E. G., Keller J. J., Offerhaus J. A., Giardiello F. M., Kleibeuker J. H. (2005): Sulindac inhibits beta-catenin expression in normal-appearing colon of hereditary nonpolyposis colorectal cancer and familial adenomatous polyposis patients. Cancer Epidemiol. Biomarkers Prev. **14,** 1608–1612

Kriajevska M., Fischer-Larsen M., Moertz E., Vorm O., Tulchinsky E., Grigorian M., Ambartsumian N., Lukanidin E. (2002): Liprin beta 1, a member of the family of LAR transmembrane tyrosine phosphatase-interacting proteins, is a new target for the metastasis-associated protein S100A4 (Mts1). J. Biol. Chem. **277,** 5229–5235

Lepourcelet M., Chen Y. N., France D. S., Wang H., Crews P., Petersen F., Bruseo C., Wood A. W., Shivdasani R. A. (2004): Small-molecule antagonists of the oncogenic Tcf/beta-catenin protein complex. Cancer Cell **5,** 91–102

Li Y., Zhang K. L., Sun Y., Yang Y., Chen X. Y., Kong Q. Y., Wu M. L., Liu J., Li H. (2008): Frequent S100A4 expression with unique splicing pattern in gastric cancers: a hypomethylation event paralleled with E-cadherin reduction and Wnt activation. Transl. Oncol. **1**, 165–176

Li Z., Bresnick A. R. (2006): The S100A4 metastasis factor regulates cellular motility via a direct interaction with myosin-IIA. Cancer Res. **66,** 5173–5180

Li Z., Spektor A., Varlamova O., Bresnick A. R. (2003): Mts1 regulates the assembly of nonmuscle myosin-IIA. Biochemistry **42,** 14258–14266

Meyskens F. L., McLaren C. E., Pelot D., Fujikawa-Brooks S., Carpenter P. M., Hawk E., Kelloff G., Lawson M. J., Kidao J., McCracken J., Albers C. G., Ahnen D. J., Turgeon D. K., Goldschmid S., Lance P., Hagedorn C. H., Gillen D. L., Gerner E.W. (2008): Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. Cancer Prev. Res. **1,** 32–38

Mizutani K., Miyamoto S., Nagahata T., Konishi N., Emi M., Onda M. (2005): Upregulation and overexpression of DVL1, the human counterpart of the Drosophila dishevelled gene, in prostate cancer. Tumori **91,** 546–551

Neth P., Ciccarella M., Egea V., Hoelters J., Jochum M., Ries C. (2006): Wnt signaling regulates the invasion capacity of human mesenchymal stem cells. Stem Cells **24,** 1892–1903

Orner G. A., Dashwood W., Blum C. A., Díaz G. D., Li Q., Dashwood R. H. (2003): Suppression of tumorigenesis in the Apc(min) mouse: down-regulation of beta-catenin signaling by a combination of tea plus sulindac. Carcinogenesis **24,** 263–267

Pathuri P., Vogeley L., Luecke H. (2008): Crystal structure of metastasis-associated protein S100A4 in the active calcium-bound form. J. Mol. Biol. **383,** 62–77

Pinto D., Gregorieff A., Begthel H., Clevers H. (2003): Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev. **17,** 1709–1713

Rajamanickam S., Kauer M., Velmurugan B., Agarwal R. (2009): Silibinin inhibits spontaneous intestinal tumorigenesis in the $APC^{min/+}$ mice model by modulating beta-catenin pathway. Proc. Am. Assoc. Cancer Res. **50,** 509

Rice P. L., Kelloff J., Sullivan H., Driggers L. J., Beard K. S., Kuwada S., Piazza G., Ahnen D. J. (2003): Sulindac metabolites induce caspase- and proteasome-dependent degradation of beta-catenin protein in human colon cancer cells. Mol. Cancer Ther. **2,** 885–892

Sablina A. A., Chumakov P. M., Kopnin B. P. (2003): Tumor suppressor p53 and its homologue p73α affect cell migration. J. Biol. Chem. **278,** 27362–27371

Schmidt-Hansen B., Klingelhofer J., Grum-Schwensen B., Christensen A., Andresen S., Kruse C., Hansen T., Ambartsumian N., Lukanidin E., Grigorian M. (2004a): Functional significance of metastasis-inducing S100A4(Mts1) in tumor-stroma interplay. J. Biol. Chem. **279,** 24498–24504

Schmidt-Hansen B., Ornas D., Grigorian M., Klingelhofer J., Tulchisnky E., Lukanidin E., Ambartsumian N. (2004b): Extracellular S100A4(mts1) stimulates invasive growth of mouse endothelial cells and modulates MMP-13 matrix metalloproteinase activity. Oncogene **23,** 5487–5495

Segditsas S., Tomlinson I. (2006): Colorectal cancer and genetic alterations on the Wnt pathway. Oncogene **25,** 7531–7537

Semov A., Moreno M. J., Onichtchenko A., Abulrob A., Ball M., Ekiel I., Pietrzynski G., Stanimirovic D., Alakhov V. (2005): Metastasis-associated protein S100A4 induces angiogenesis through interaction with annexin II and accelerated plasmin formation. J. Biol. Chem. **280,** 20833–20841

Senolt L., Grigorian M., Lukanidin E., Simmen B., Michel B. A., Pavelka K., Gay R.E., Gay S., Neidhart M. (2006): S100A4 is expressed at site of invasion in rheumatoid arthritis synovium and modulates production of matrix metalloproteinases. Ann. Rheum. Dis. **65,** 1645–1648

Shan J., Shi D., Wang J., Zheng J. (2005): Identification of a specific inhibitor of the dishevelled PDZ domain. Biochemistry **44,** 15495–15503

Silva A., Bodyak N., Sauer N., Vaze M., Laroux S., Harboth J., Li C. J., Fruehauf J. H. (2009): Safety and efficacy of a tkRNAi therapeutic targeting beta-catenin (CTNNB1). Proc. Am. Assoc. Cancer Res. **50S,** 39

Sporn M. B., Hong W. K. (2008): Concomitant DFMO and sulindac chemoprevention of colorectal adenomas: a major clinical advance. Nat. Clin. Pract. Oncol. **5,** 628–629

Stein U., Arlt F., Walther W., Smith J., Waldman T., Harris E. D., Mertins S. D., Heizmann C. W., Allard D., Birchmeier W., Schlag P. M., Shoemaker R. H. (2006): The metastasis-associated gene S100A4 is a novel target of β-catenin/T-cell factor signaling in colon cancer. Gastroenterology **131,** 1486–1500

Stein U., Schlag P. M. (2007): Clinical, biological, and molecular aspects of metastasis in colorectal cancer. Recent Results Cancer Res. **176,** 61–80

Takenaga K., Nakamura Y., Sakiyama S., Hasegawa Y., Sato K., Endo H. (1994): Binding of pEL98 protein, an S100-related calcium-binding protein, to nonmuscle tropomyosin. J. Cell Biol. **124,** 757–768

Taniguchi H., Yamamoto H., Hirata T., Miyamoto N., Oki M., Nosho K., Adachi Y., Endo T., Imai K., Shinomura Y. (2005): Frequent epigenetic inactivation of Wnt inhibitory factor-1 in human gastrointestinal cancers. Oncogene **24,** 7946–7952

Tuma R. (2008): Drugs to prevent colon cancer show promise, but hurdles remain for chemoprevention. J. Natl. Cancer Inst. **100,** 764–766

Tuynman J. B., Vermeulen L., Boon E. M., Kemper K., Zwinderman A. H., Peppelenbosch M. P., Richel D. J. (2008): Cyclooxygenase-2 inhibition inhibits c-Met kinase activity and Wnt activity in colon cancer. Cancer Res. **68,** 1213–1220

Uematsu K., He B., You L., Xu Z., McCormick F., Jablons D. M. (2003b): Activation of the Wnt pathway in non small cell lung cancer: evidence of dishevelled overexpression. Oncogene **22,** 7218–7221

Uematsu K., Kanazawa S., You L., He B., Xu Z., Li K., Peterlin B. M., McCormick F., Jablons D. M. (2003a): Wnt pathway activation in mesothelioma: evidence of dishevelled overexpression and transcriptional activity of β-catenin. Cancer Res. **63,** 4547–4551

Vallely K. M., Rustandi R. R., Ellis K. C., Varlamova O., Bresnick A. R., Weber D. J. (2002): Solution structure of human Mts1 (S100A4) as determined by NMR spectroscopy. Biochemistry **41,** 12670–12680

Vincan E., Barker N. (2008): The upstream components of the Wnt signalling pathway in the dynamic EMT and MET associated with colorectal cancer progression. Clin. Exp. Metastasis **25,** 657–663

Watanabe Y., Usada N., Minami H., Morita T., Tsugane S., Ishikawa R., Kohama K., Tomida Y., Hidaka H. (1993): Calvasculin, as a factor affecting the microfilament assemblies in rat fibroblasts transfected by src gene. FEBS Lett. **324,** 51–55

Yammani R. R., Carlson C. S., Bresnick A. R., Loeser R. F. (2006): Increase in production of matrix metalloproteinase 13 by human articular chondrocytes due to stimulation with S100A4: Role of the receptor for advanced glycation end products. Arthritis Rheum. **54,** 2901–2911

Yoon C. S., Hyung W. J., Lee J. H., Chae Y. S., Won N. H., Yeom B. W., Choi J. S. (2008): Expression of S100A4, E-cadherin, alpha- and beta-catenin in gastric adenocarcinoma. Hepatogastroenterology **55**, 1916–1920

You L., He B., Xu Z., Uematsu K., Mazieres J., Fujii N., Mikami I., Reguart N., McIntosh J. M., Kashani-Sabet M., McCormick F., Jablons D. M. (2004): An anti-Wnt-2 monoclonal antibody induces apoptosis in malignant melanoma cells and inhibits tumor growth. Cancer Res. **64,** 5385–5389

You L., Xu Z., Punchihewa C., Jablons D. M., Fujii N. (2008): Evaluation of a chemical library of small-molecule Dishevelled antagonists that suppress tumor growth by down-regulating T-cell factor-mediated transcription. Mol. Cancer Ther. **7,** 1633–1638

Zhang Y., Appleton B. A., Wiesmann C., Lau T., Costa M., Hannoush R. N., Sidhu S. S. (2009): Inhibition of Wnt signaling by Dishevelled PDZ peptides. Nat. Chem. Biol. **5,** 217–219

Received: May 15, 2009
Accepted: June 10, 2009