Gastrointestinal cancer - only a deregulation of stem cell differentiation? (Review)

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Abstract. Recent research on embryonic and adult stem cells questions the currently accepted models of multi-step carcinogenesis in solid cancer. Accordingly, differentiated epithelial cells are considered to be the main target for mutational steps, leading to a growth and survival advantage of malignantly transformed cells. In contrast, the stem cell model of carcinogenesis emphasizes the role of stem cells as the initiating structure for tumor development. Yet, it is unclear if tumors contain dysregulated (embryonic) stem cells or if tumors consist of differentiated adult cells that obtained a de-differentiated stem cell-like phenotype. Here, we review the current knowledge on the roles of stem cells in gastrointestinal cancer formation and the implication on future diagnostic and therapeutic strategies.

Contents

1. Introduction

Gastrointestinal malignancies are among the most frequent cancer diseases in men and women and represent leading causes of cancer-related deaths worldwide (1,2). Especially colorectal (3,4), liver (5) and pancreatic cancers (6) still remain urgent medical problems. Although some progress has been made in treatment of early disease stages, advanced and metastasized carcinomas can only be treated palliatively. Several risk factors like infectious agents (e.g. Hepatitis C virus), chronic inflammation (e.g. colitis ulcerosa or chronic pancreatitis) or genetic predispositions (e.g. familial forms of colorectal cancer such as familial adenomatous polyposis) have been identified in the past. Additionally, different genetic changes like activation of oncogenes as well as genetic or epigenetic inactivation of tumor suppressor genes were commonly observed in several cancer types, leading to the postulation of the so-called multistep carcinogenesis models (7,8). While these models are attractive in supporting morphologic and molecular diagnosis at early stages, they fail to explain several commonly observed features of tumor development and progression, dissemination or relapse.

2. The current concept: multistep carcinogenesis

The present textbook knowledge of tumor development favors the consecutive accumulation of oncogenic events in preneoplastic cells that finally leads to a malignant phenotype. In these models, several activating mutations in cellular proto-oncogenes as well as the genetic or epigenetic inactivation of tumor suppressor genes lead to a stepwise alteration of the morphologic appearance of normal epithelium to dysplasia, adenoma or intraepithelial premalignant lesions, and finally to invasive carcinoma (Fig. 1). Several distinct oncogenic pathways are commonly altered during the course of this development, e.g. changes in ß-catenin/Wnt-signaling, inactivation of endogenous cell cycle regulators such as Rb or $p16^{ink4a}$, re-activation of telomerase or mutations in K-ras or p53. These genetic or epigenetic events lead to the acquisition of growth promoting features defined as the 'hallmarks of cancer' by Hanahan and Weinberg in their outstanding review (9). In particular, these include: 1) the independence of the tumor cell to growth-regulating signals (e.g. constitutive activation of K-ras by point mutations in codon 12; mutations in p53 or Rb) (10-18), 2) deficiency in apoptosis (e.g. overexpression of bcl-2) (19-24), 3) tissue remodeling and metastasis (e.g. ß-catenin/Wnt) (25-29), 4) unrestricted proliferation (activation of telomerase) (30-32) and 5) induction of neo-angiogenesis (e.g. by autocrine or

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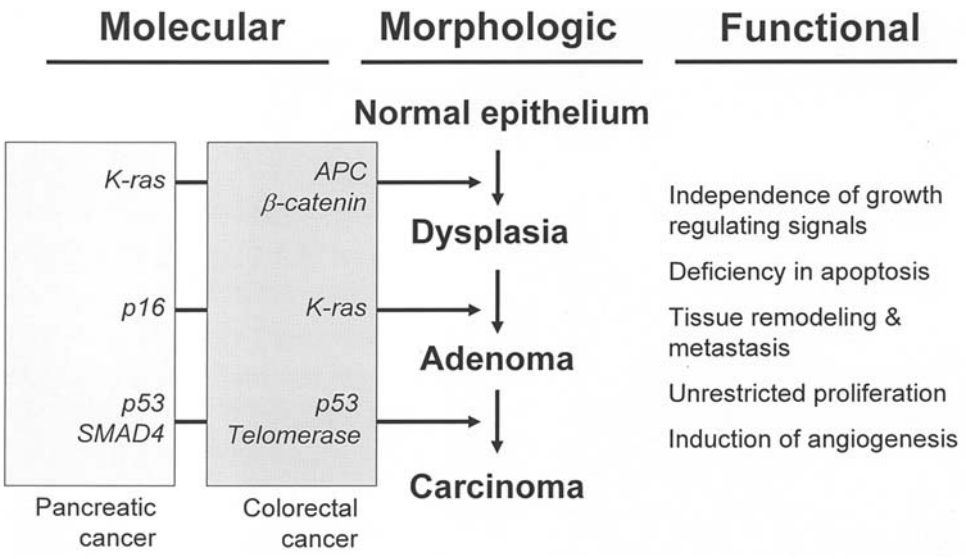

Figure 1. Model for multistep carcinogenesis in colorectal and pancreatic cancer development. Modified schematic overview over the current models of colorectal (7) and pancreatic (8) cancer development. Several oncogenic events are acquired during malignant transformation which provide a survival and growth advantage for the transformed cell, leading to the functional state of a cancer cell (9). The genetic events depicted here are variable in different tumor entities and some of the morphologic or genetic features can also be omitted during tumor development.

paracrine secretion of VEGF) (33-35). It is important to note that during this process of malignant transformation not all of these steps have to be fulfilled and that there is a huge variety of possibilities for the timing and the order of the above described genetic or epigenetic changes. Additionally, these multistep models should not be considered as a linear process but should be viewed as a non-linear dynamic network of intertwining and interdependent steps of malignant transformation (36,37).

3. Constraints of multistep models

The acquisition of several mutations that synergistically provide the epithelial cell with a growth advantage is of low statistical probability as the differentiated epithelium in the gastrointestinal tract is predominantly in the G_0 state of the cell cycle. Most of the genetic material is present as tightly packed and highly organized chromatin in the center of the nucleus, thus being protected from mutational influences, e.g. by irradiation or chemical carcinogens. Additionally, most genetic programs regulating cell growth and migration are inactivated during differentiation and maturation (38-40) and can be rescued by numerous redundant pathways. Gastrointestinal epithelia, especially of large and small intestine, are regenerated by asymmetric division of peripheral stem cells at the crypt basis, giving rise to a small number of highly proliferating progenitor cells that migrate and differentiate to terminal epithelial cells in a course of 2-3 days. Overall, the life-time of differentiated epithelial cells is limited to about 7-10 days in humans, which further decreases the statistical probability for acquiring targeted mutations (41-43).

Recent research efforts therefore focused 1) on the role of long-living and persistent stem and progenitor cells during carcinogenesis, an already accepted concept for hematopoietic neoplasias (44-46), but 2) also on the possibility of trans- or de-differentiation of mature cells during carcinogenesis (44,47-49).

4. Stem cells in the gastrointestinal system

The organs of the gastrointestinal system possess a high capability of continuous tissue regeneration (e.g. mucosa of small and large intestine) or in response to acute or chronic injuries or inflammatory conditions (e.g. chronic helicobacter pylori gastritis; inflammatory bowel diseases, such as colitis ulcerosa; chronic viral hepatitis). These processes are largely maintained by a stable pool of peripheral stem cells that are tightly regulated in their proliferative capacity and give rise to a pool of highly proliferating progenitor cells (42,43,50). These progenitor cells already lost some of the key features of true stem cells, predominantly the capabilities to asymmetric self-renewal and limitless replication with extended life span. Intestinal stem cells are phenotypically characterized by nuclear expression of ß-catenin (51) and of the RNA-binding protein Musashi-1 (52-54). These factors are involved in Wnt/APC- and Notch-signaling, respectively, which have also been shown to be important pathways for colorectal carcinogenesis (29,55-58). Some other factors identified in stem cell differentiation and carcinogenesis are Oct-4, Notch, bone morphogenic protein (BMP), janus family kinase (JAK) or sonic hedgehog (Shh) (9,44,58-60) in different anatomic gastrointestinal sites indicating the close relationship between the tightly regulated physiologic process of stem cell maintenance and differentiation and the dysregulated malignant transformation. During embryonic development, but also during the process of cell maturation and migration from the colonic crypt basis, stem cells and their offspring are able to induce angiogenesis and matrix remodeling (60).

A population of progenitor cells has been identified in different forms of liver regeneration and has also been associated with the development of hepatocellular carcinomas. These so-called oval cells form a distinct population in the periductular region of the liver lobules and express markers specific for hepatocytes (e.g. AFP, albumin), as well as biliary tract markers such as cytokeratin 19 or γ-GT, and

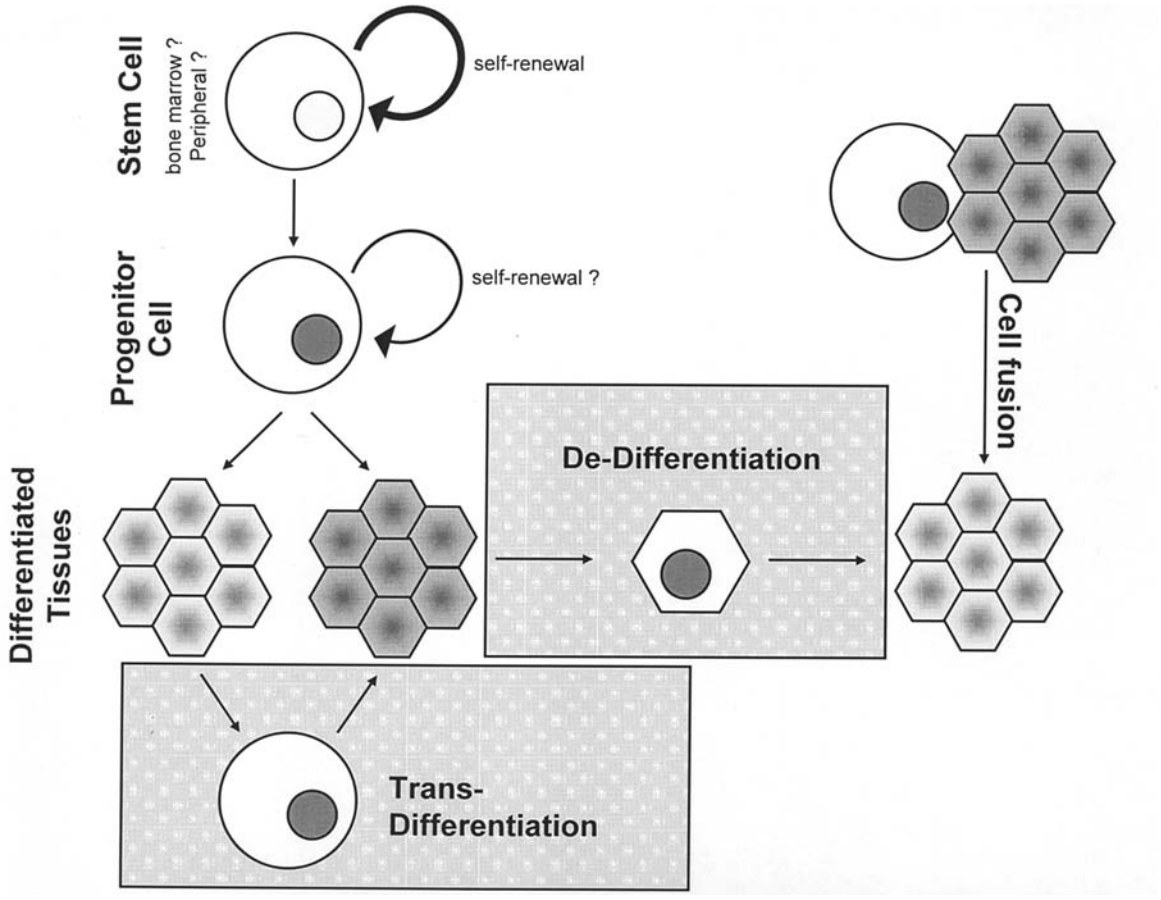

Figure 2. Tissue and stem cell plasticity. True stem cells have the capability of self-renewal and asymmetric division into a resting stem cell and an already committed progenitor cell, which can either give rise to one or to multiple cell types, and has only a limited capacity of self-renewal. Trans-differentiation describes the re-programming of progenitor cells to form different cell types or tissues, while de-differentiation denotes the acquisition or re-activation of immature (stem or progenitor) cell capabilities to give rise to new phenotypes. Adapted from (49,60).

classical bone marrow markers like Thy-1, CD34, c-kit or flk-2 (61). In addition to this phenotypic characterization, experimental evidence from rodents and humans shows that bone marrow cells are capable of repopulating the liver (62-64) and that these progenitor cells are involved in different liver diseases such as hepatocellular carcinoma or cirrhosis (65-69). The notion of bone marrow-derived cells contributing to peripheral tissue maintenance in the gastrointestinal tract has been strengthened by observations of female patients receiving a mixed-sex bone marrow transplantation from male donors. Differentiated Y-chromosome-positive cells have been detected in various organs of the gastrointestinal tract (64,70,71). Yet, the exact mechanism of how bone marrow-derived cells support peripheral tissue regeneration is still under debate and several models (e.g. cell fusion, trans-differentiation, de-differentiation, phenocopying) are controversially discussed in the literature (47,60,72) (Fig. 2).

5. Contribution of stem (cell-like) cells in gastrointestinal carcinogenesis

Comparing the properties of cancer cells and (embryonic) stem cells, there seem to be more similarities than differences and of course, more similarities than to resting and differentiated adult cells (Fig. 3). Therefore, two main questions arise: 1) Do stem cells really participate in tumorigenesis and 2) are these cells true stem cells or de-differentiated adult cells with a temporary stem cell-like phenotype?

Morphological comparisons between gastrointestinal embryogenesis and carcinogenesis displayed a similar patterning, i.e. organized arrangement of cells and tissues. In the colon, embryologic development is characterized by the buildup of primitive tubules. Adenomas and adeno-carcinomas of the colon show the same pattern with tubular branching imitating colon embryology in an apparently uncontrolled fashion (73,74).

These findings are further supported by the observation that relevant markers of embryogenesis are expressed in early or late stages of gastrointestinal tumorigenesis. While cytokeratin 7 (CK7) is abundantly expressed in the fetal stomach, it is barely detectable in adult gastric tissue. Yet, during gastric carcinogenesis, a neo-expression of CK7 is observed. These cells are considered as de-differentiated cells resembling a stem cell-like phenotype (75). Similar results regarding the nuclear expression of ß-catenin were obtained from colorectal adenomas (73). In pancreatic cancer development, re-expression of the pancreatic duodenal homeobox gene 1 (PDX-1), a regulator of exocrine/endocrine development during embryogenesis, is commonly observed (76,77). A further regulator of embryonic gut development is the family of Hedgehog (Hh) proteins that is required for correct specification and patterning (78). Especially, expression

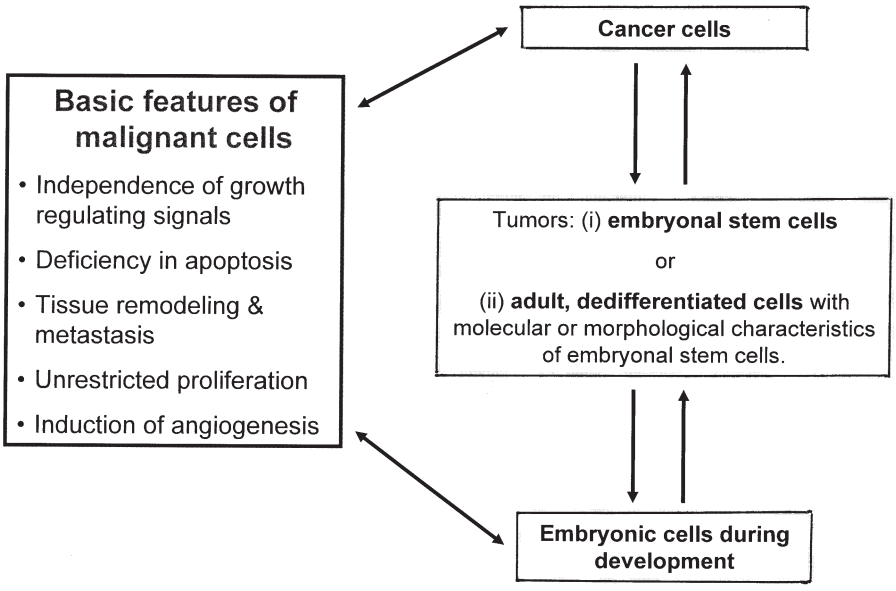

Figure 3. Schematic comparison of tumor and stem cell properties. Both cell types posses growth-promoting features that distinguish them from terminally differentiated cells. It is thus conceivable that tumors either harbor a stem cell population with reactivated embryonic properties or that epithelial cells dedifferentiate to a more stem cell-like phenotype by the acquisition of several genetic or epigenetic events.

of Sonic Hedgehog (Shh) has been attributed to gastrointestinal stem cells and its overexpression has now been described in colorectal, pancreatic and other digestive tract tumors (79-83). The expression of early markers of hepatic development has been observed in dysplastic foci of the liver and in hepatocellular adenomas. This has been linked to the presence of hepatic progenitor cells or the fusion of progenitor cells with mature hepatocytes, but not to the reactivation of embryonic programs in differentiated liver parenchyma (84-86).

Recently, a novel class of non-coding RNAs, the so-called micro-RNAs (miRNA), have been identified as important regulators of gene expression, cellular differentiation and survival (87,88) and might be involved in tumorigenesis by dysregulating oncogenes such as ras (89-95). However, the exact meaning and impact of miRNAs on stem cell biology, differentiation, and cancer development remains to be clarified.

In summary, the processes of determination in embryonic differentiation as well as in gastrointestinal carcinogenesis are relevantly influenced by various signaling pathways (e.g. Wnt/ß-catenin, sonic hedgehog proteins). Additionally, morphology studies showed major morphological similarities and analogies of these two scenes. Overall, tumor cells display morphological phenotypes and molecular markers of early embryonic development with the complete, possibly dangerous, potency of this de-differentiation status. Besides the classical hallmarks of cancer such as proliferation, apoptosis, tissue remodeling and metastasis as well as induction of neoangiogenesis, it seems that de-differentiation and re-activation of embryonic-signaling pathways plays an additional role in gastrointestinal carcinogenesis. With reference to our questions at the beginning of this paragraph, we suggest that de-differentiation processes are essentially involved in this process, as it is not possible to distinguish embryonic from adult stem cells with our currently established techniques. Several studies revealed a relevant and reciprocal inter-acting association between the hallmarks of malignancy, treatment outcome and patient survival with markers of differentiation, e.g. the Wnt/ß-catenin pathway (96-98), which could be used for novel targeted approaches.

6. Modulation of differentiation status of gastrointestinal tumors

Presuming that human gastrointestinal tumors resemble de-differentiated states of embryonic or adult stem cells, it will be an interesting therapeutic approach to induce differentiation of these tumor cells into normal resting adult cells or to reduce the malignant potency of de-differentiation.

Several classes of differentiation modulating agents have been examined and tested in pre-clinical or clinical settings. Among these, natural or synthetic derivatives of retinoic acids (e.g. all-trans retinoic acid, ATRA), epigenetic modulators such as the DNA methyltransferase inhibitor zebularine, inhibitors of histone deacetylases like suberoylanilide hydroxamic acid (SAHA) or Trichostatin A (TSA), as well as 'specific' inhibitors of WNT/ß-catenin or hedgehog-signaling-like cyclopamine, are the most prominent. Besides inhibition of proliferation and induction of apoptosis, retinoids interact with nuclear receptors forcing differentiation of cells in several non-gastrointestinal malignancies such as acute promyelocytic leukemia, teratocarcinomas and different solid tumors (e.g. squamous cell carcinoma and breast carcinoma) (99). Several natural and synthetic derivatives are currently tested in clinical trials. Additionally, retinoids have the potential of chemoprevention (100). Epigenetic modulators (e.g. zebularine or SAHA) regulate gene transcription via inhibition of DNA methylation or deacetylation of lysine residues in core histones. Hypermethylation and hypoacetylation are observed in many solid tumors, especially in gastrointestinal tumors. These phenomena have been linked

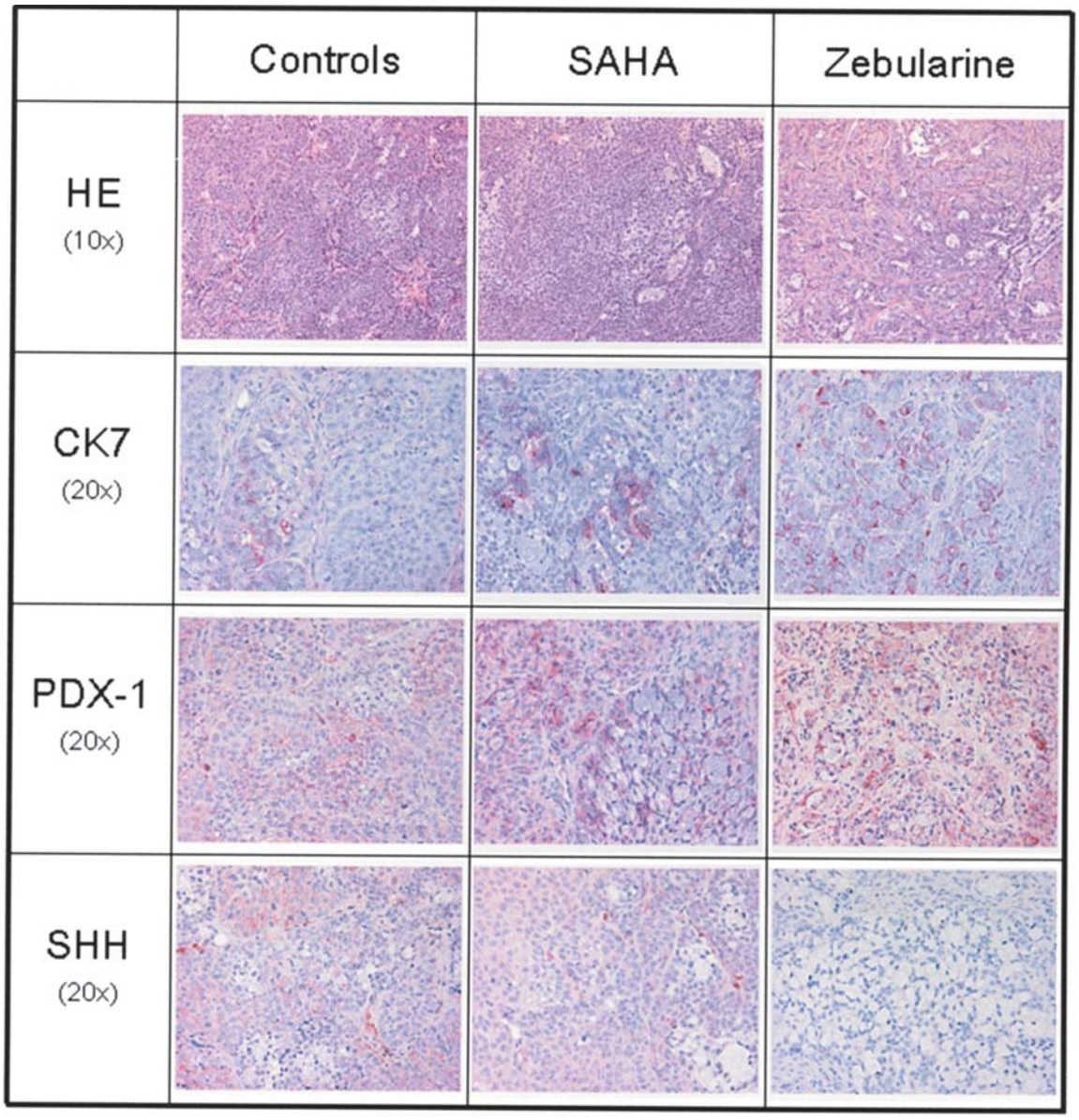

Figure 4. Morphologic and molecular stabilization of pancreatic cancer xenografts after treatment with epigenetic modulators. Tumor-bearing nude mice were treated intraperitoneally with the DNMT inhibitor zebularine or the HDAC inhibitor SAHA and parameters of differentiation [cytokeratin (CK) 7] and embryonic-signaling pathways (pdx-1 and shh) were assessed by immunohistochemistry in paraffin-embedded specimens as described (77; unpublished data) compared to untreated controls. In short, our experiments show a morphological shift from a solid (controls) to a more ductal phenotype (zebularine, H&E-staining), associated with an upregulation of CK7 and PDX-1 and a downregulation of SHH.

to the inactivation of tumor suppressor genes (e.g. transcriptional repression of $p16^{ink4a}$ by promoter hypermethylation), while the inactivation of genes by these processes is also commonly observed during embryogenesis and cellular differentiation. *In vitro* and *in vivo* experiments confirmed that these two classes of drugs (inhibitors of DNA methylation and histone deacetylation) have anti-proliferative and pro-apoptotic capabilities as well as pro-differentiation potency (101-104). Our experience with zebularine and SAHA confirmed earlier findings, that these compounds have anti-proliferative and pro-apoptotic effects (105). Additionally, pancreatic carcinoma xenografts in nude mice show a morphological and molecular stability after this treatment, especially regarding the expression of different cytokeratins or PDX-1 (Fig. 4) (77; unpublished data). Different agents (e.g. cyclopamine or sulindac) have been identified which selectively inhibit components of the Wnt/ß-catenin or hedgehog signaling pathways; thus, inhibiting proliferation and inducing apoptosis and differentiation (106). These results indicate that interference with these embryonic pathways, which represent early changes during the process of carcinogenesis, might be promising approaches for the development of future therapies.

7. Summary and future directions

The challenge is to gain better understanding and deeper knowledge on mechanisms and impact of differentiation and its dysregulation in the process of malignant transformation of embryonic or adult stem cells. As current therapy options aim at tumor cells at the end of a differentiation process, novel therapies should concentrate on the other side of this scale, i.e. early changes in stem cell-like tumor progenitor cells that

could revert the instability of the differentiation status and lead to a phenotypic stabilization.

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