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TABLE OF CONTENTS

Zusammenfassung

Krebs und Differenzierung sind eng verwandte biologische Phänomene. Um molekulare Abläufe zu erforschen und an Krebsdifferenzierung beteiligte Gene zu entdecken, haben wir ein in vitro Modell entwickelt, dass die Induktion der Differenzierung in Lungenkrebszelllinien ermöglicht. Mit diesem Modell konnten wir Gene charakterisieren, die nach Induktion der Differenzierung hochreguliert werden. Die kleinzellige Lungenkarzinomzelllinie (SCLC) H526 und die nicht-kleinzellige Lungenkarzinomzelllinie (NSCLC) H2228 wurden mit der Differenzierung induzierenden Chemikalie 5-Bromodesoxyuridin (BrdU) behandelt. Die Behandlung mit BrdU führte in H526 zu einer dramatischen morphologischen Transformation, einer bemerkenswert reduzierten Fähigkeit zur Koloniebildung in weichem Agar und verringerter Tumorwachstumsrate in Nacktmäusen. Die Behandlung von H2228 mit BrdU führte ebenfalls zu sichtbarer Wachstumsverringerung der Zellen. Nach erfolgreicher Etablierung des Zellmodells konnte die Expression von drei Gengruppen beobachtet werden, darunter Wachstumsfaktoren wie das Insulinwachstumsfaktor-bindende Protein 7 (IGFBP-7), Transkriptionsfaktoren wie der „paired-like“ Homöodomain Transkriptionsfaktor 1 (PITX1), Lungenkrebs-assoziiertes Gen Y (LAGY), Homöobox B2 (HoxB2) ebenso wie extra- und intrazelluläre Adhäsionsmoleküle, darunter Connexin 26 (Cx26), Laminin Alpha 3 und Epitheliales V-ähnliches Antigen 1 (EVA1). Die genannten Gene waren erheblich hochreguliert auf mRNA und/oder Proteinebene. Wir untersuchten zusätzlich die Mechanismen, die der differenziellen Expression von IGFBP-7 zugrunde liegen und fanden heraus, dass eine abweichende Methylierung der DNA mit einer Verringerung der Expression von IGFBP-7 sowohl in Darmals auch in Lungenkrebs einhergeht, was auf einen Zusammenhang zwischen abweichender Genmethylierung und Krebsdifferenzierung schließen lässt.

Summary

Title

Characterization of genes involved in cancer differentiation

Abstract

Cancer and differentiation are closely related biological phenomena. In order to investigate molecular events and to discover genes involved in the cancer differentiation, we developed an *in vitro* model that induced differentiation in lung cancer cell lines and characterized associated genes being up-regulated upon induction of differentiation. The small cell lung carcinoma (SCLC) cell line H526 and the non-small cell lung carcinoma (NSCLC) cell line H2228 were treated with the differentiation inducing agent 5-bromodeoxyuridine (BrdU). The BrdU treatment in H526 led to a dramatic morphological transformation and remarkably reduced the ability of colony formation in soft agar and suppressed the tumor growth rate in nude mice. Similarly, the treatment of H2228 led to the obvious growth suppression of the cells. After the successful construction of the cell model, the expression of three categories of genes could be observed covering growth factors like the insulin-growth factor binding protein 7 (IGFBP-7), transcription factors such as paired-like homeodomain transcript factor 1 (PITX1), lung cancer associated gene Y (LAGY), homeobox B2 (HoxB2), as well as extra- and intra-cellular adhesion molecules including Connexin 26 (Cx26), Laminin alpha 3 and epithelial V-like antigen 1 (EVA1). All these genes were remarkably up-regulated at the mRNA and/or protein level. In addition, we studied the mechanisms underlying the differential expression of IGFBP-7 and found that aberrant DNA methylation was closely related to the downregulation of IGFBP-7 in both colon cancer and lung cancer suggesting an association between aberrant gene methylation and cancer differentiation.

Introduction

Differentiation is a highly relevant topic in tumor pathology for two reasons. First, it describes the description and definition of specific cell types that may give rise to cancer. Together with the dignity, it is thus of utmost importance in tumor classification. Second, it is used to define the histological grade of a tumor which refers to a system used to classify cancer in terms of how much the tumor cells resemble normal cells of the same tissue type (http://www.cancer.gov/cancertopics). According to the extent of differentiation, tumors are scored as poorly, moderately or well differentiated corresponding to low grade (G1), intermediate grade (G2) or high grade (G3) neoplasias, respectively. Aberrant differentiation is thus a basic characteristic of tumors which are additionally characterized by unlimited cell proliferation and other features of autonomous cell growth. In contrast normal terminal differentiation is associated with cell maturation and growth arrest. Differentiation-associated genes should therefore harbor characteristics of tumor suppressors. Many drugs such as nerve growth factor, all trans retinoic acid, active form vitamin D (3), transforming growth factor-beta, cAMP and 5-bromodeoxyuridine are known to have a differentiation-induced capability on solid tumors *in vitro* and/or *in vivo* [1,2].

Lung cancer is the leading cause of cancer death in the United States, with 162,000 deaths expected in 2006. Worldwide, this corresponds to 1,000,000 cancer deaths annually. The prognosis of the disease is very poor with an overall 5-year survival rate of 15%, a proportion that compares poorly with the mortality rates of 99 %, 88 %, and 64% of prostate, breast, and colorectal carcinoma, respectively [3,4]. Due to its high incidence colorectal cancer remains the third and second leading cause of death in male and female respectively in the US, where it affected more than 148,000 individuals in 2006 [3]. Histologically, colorectal carcinomas are almost exclusively adenocarcinoma, treatment is primarily determined by clinical stage, at times modified by results of molecular assays. In contrast, the classification of lung cancer is more complicated as evidenced by the WHO classification. The major distinction is between NSCLC which represent about 80% of cases being essentially divided into squamous cell carcinoma, adenocarcinoma, large cell carcinoma and mixed types, and SCLC which

represent about 20% of lung cancers [5].

The advances of molecular approaches in cancer research may have direct relevance to the goals of early detection, accurate prognostic assessment, and targeted therapy. Subtractive Suppression Hybridization (SSH) is a classical and useful method to discover differentially expressed genes [6]. To discover lung cancer associated genes, our lab previously constructed cDNA libraries by Suppression Subtraction Hybridization (SSH) and examined the global gene expression profiling in human lung cancers by using a 24,000 element cDNA microarray [7-10]. Thereby, we identified several differentiation-associated genes being of potentially involved for lung carcinogenesis [11,12].

Differentiation-associated genes can potentially induce terminal differentiation in tumor cells and subsequent cell death. Defect in these genes may instead induce continuous proliferation, the formation of preneoplastic and finally neoplastic lesions [13]. Although the involvement of differentiation in carcinogenesis has been proposed [14,15], differentiation-associated genes are not yet well understood and analyzed in the process of carcinogenesis. Kedar et al. proposed that downregulation of differentiation-associated genes may be the primary event in human carcinogenesis [13]. The molecular analysis of cancer differentiation in cell models could reveal important findings for tumor ontogeny as well as for the targeted therapy of malignant tumors.

Objectives

In order to investigate the molecular events and to characterize of genes underlying cancer differentiation we aimed at the following objectives:

1. The development of an *in vitro* model that can induce differentiation in lung cancer cell lines.

2. The analysis of differentiation associated genes on the mRNA and/or protein levels in the cell model

3. The more in depth characterization of the gene IGFBP-7 gene being differentially expressed in lung and colon cancers

Materials and methods

Cell lines and tumor specimens

The cancer cell lines were either purchased from the American Tissue Type culture collection (ATCC, Rockville, MD) or from the German Collection of Microorganisms and Cell Culture (DSMZ, Braunschweig, Germany). In addition single lung cancer cell lines were established by our group [7]. The cell lines were cultured in proper mediums as described [7,9,10,16,17].

Development and analysis of the cancer differentiation model

Suspension of the SCLC cell line H526 and adherent NSCLC cell line H2228 was exposed to the classic differentiation inducer 5-bromodeoxyuridine (BrdU). DNA fingerprinting was performed to prove that the parental and BrdU-treated cell lines were isogenic [18,19].

The treated and untreated cell lines were analyzed *in vitro* for tumor cell growth curve by standard proliferation assay and anchorage-independent growth by soft agar test as described [19]. The ability to form tumors was assessed by the inoculation of tumor cells into nude mice, a classical *in vivo* tumorigenicity assay [19].

Tumor specimens and tissue microarray construction

Primary tumor specimens originated from cancer patients that were operated at the Charité university hospital. Tissue microarrays (TMA) were constructed from paraffin tissue blocks for immunohistochemistry (IHC). In addition, fresh frozen material was available for expression analysis by RT-PCR [16,17,20].

Expression analysis

Expression analysis of the cell lines was performed by RT-PCR and/or Northern blot as well as Western blot. Primer information for RT-PCR was referred in Table 1. Primary tumor samples were assessed by RT-PCR and IHC.

Table 1 Primers used for RT-PCR

Gene	Sense (5'→3')	Antisense (5'→3')	Annealing Temp.	Product size	Source
IGFBP-7	CATCACCCAGGTCAGCAA	TGGAGGTTTATAGCTCGG	52°C	417 bp	Genbank accession no. NM_001553
LAGY	TGCTAGCTGTCCTGCTGT	GTTTCTGTCTTCTGGCCC	53°C	478 bp	Chen et al. (2003)
PITX1	CGTACGCACTTCACAAGC	AAGGTGAAGCTCTTGGTG	52°C	326 bp	Genbank accession no. NM_002653
CX26	CATCCGGCTATGGGCCCT	CAGTGACATTCAGCAGGATG	56°C	407 bp	Genbank accession no. NM_004004
Laminin alpha 3	TGCCATTTCTTCAGCCTC	TTCTTGGTTTATGCAGTC	50°C	497 bp	Genbank accession no. NM_000227
EVA1	ATGGGACAGATGCTCGGTTAA	AGGAAGTGGATCTCAGAGAAG	58°C	345 bp	Genbank accession no. NM_005797

Mechanism analysis of downregulation of IGFBP-7 in colon and lung cancers

The methylation status of IGFBP-7 was evaluated by sequencing of the PCR products of bisulfite modified genomic DNA [17,20].

Statistical analysis

The statistical analysis was done using the statistical software package SPSS. P value <0.05 was considered statistically significant.

Further details of the Materials and Methods are available in the attached publications.

Results and discussion

Induction of differentiation of lung cancer cells by treatment with BrdU

5-bromodeoxyuridine (BrdU) is a classical differentiation inducer and has been used to induce differentiation in a variety of neuroendocrine tumor cell lines including melanoma, neuroblastoma and small cell lung carcinoma [21,22]. In our study, the SCLC cell line H526 and the NSCLC cell line H2228 were treated with this agent. The morphologiccal transformation and the reduction of tumor growth supported the successful construction of the model which was particularly evident for the cell lines H526.

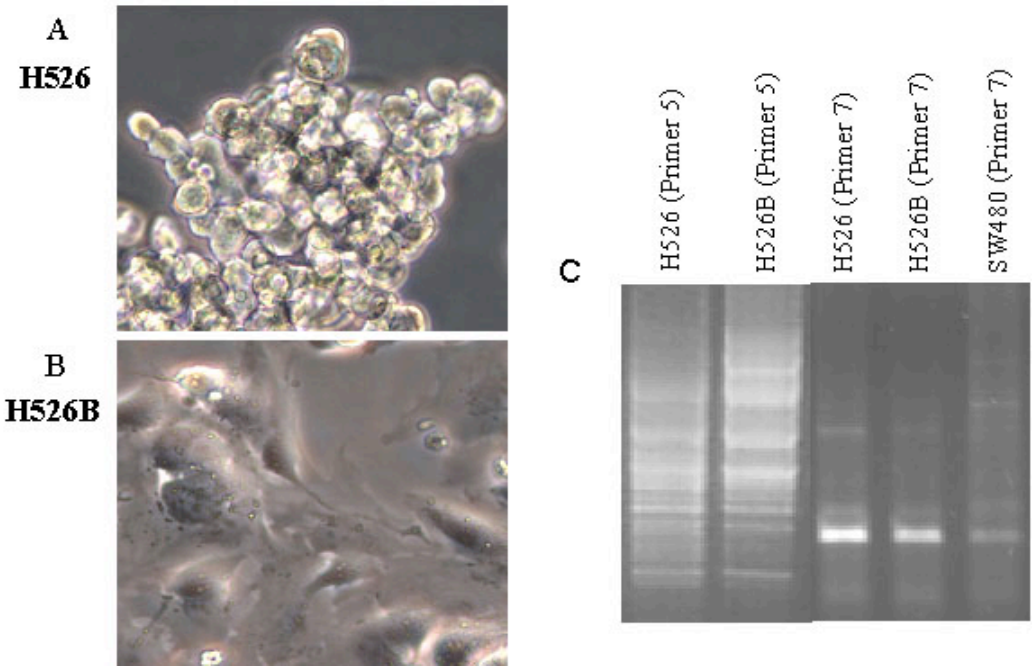

Fig. 1 Morphological characterization of the SCLC cell line H526 and its derivate H526B. H526 cells were treated with BrdU to develop the stable adherent H526B cell line. (A) The phase contrast micrograph showed H526 cells that aggregated in suspension. (B) Phase contrast micrograph showing the epitheloid morphology of the H526B cell monolayer. (C) DNA fingerprinting of these two cell lines showed the same DNA banding patterns being different from SW480 serving as control.

After 8-week BrdU treatment the parental cell line consisting of floating cells was converted into a cell population (H526B) showing characteristics of an adherently growing cell line (Fig.

1A and 1B). A pronounced phenotypic difference between the cluster-formation of H526 and the substrate-adherent monolayer of H526B was observed. H526B retained its adherent growth even in the absence of BrdU. DNA fingerprinting demonstrated for all primer sets an identical DNA banding pattern of H526 and H526B being distinct from the PCR amplification pattern of the human colon cancer cell line SW480 which served as control (Fig 1C). The data indicated that the H526B cell clone originated from the original cell line H526 and was not a contamination from other cell types.

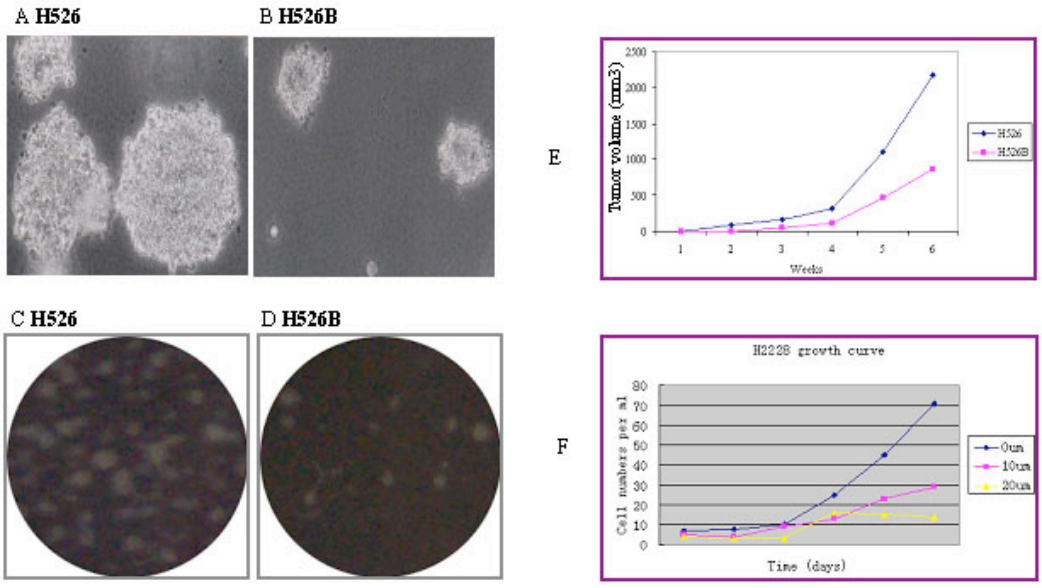

Fig. 2 Electron microscopical images of H526 (A) and H526B (B). The BrdU modified cell line H526B had a reduced ability to form colonies in soft agar compared to the parental cell line H526: Images from the visual inspection of H526 (C) and H526 B (D). BrdU modified H526B cells had a reduced tumor growth rate in nude mice compared to the parental cells of H526 (E). The proliferation of NSCLC cell line H2228 was inhibited after BrdU treatment compared to the untreated cells (F)

In the soft agar test, the colony-forming capacity of the BrdU-treated H526B cells was dramatically suppressed compared to the original cells H526. After 4 weeks of incubation in soft agar, fewer colonies as well as smaller sizes of the colonies were observed for H526B compared to H526 under the light microscope (Figs.2 A and B) and by direct ophthalmic observation (Fig.2 C and D). The tumorigenicity assay revealed that mice given injections of

H526B showed remarkably suppressed tumor growth rates compared to those receiving an injection of H526 (Fig. 2E). Compared to its original cell line H526, the derivate H526B acquired a stable and well-differentiated epithelial phenotype [19].

The morphological observation that following removal of BrdU a stable epithelial phenotype was observed in H526B suggested that the differentiation state was a cellular response being dependent on alterations in gene expression [23]. It was previously demonstrated that the phenotypic transition was associated with a decrease in the expression levels of the mesenchymal marker vimentin and the neural cell adhesion molecule (NCAM) and an increased expression of epithelial differentiation markers such as cytokeratins, cadherins and catenins [2]. To further characterize the H526 cell model, firstly, two classical lung differentiation markers, thyroid transcription factor 1 (TTF1) and surfactant protein C (SFTPC), were selected for expression analysis. TTF1 is a member of the NKX2.1 gene family consisting of homeodomain-containing transcriptional factors that are required for the early lung organogenesis. TTF-1 activates transcription of target genes including the surfactant protein critical for lung function [24,25]. An elevated protein expression of both TTF-1 and SFTPC was observed by Western blot in the BrdU-modified cell line H526B (Fig.4 A and B), providing further evidence that the adherent cells represent a better-differentiated state than the parental cells growing in suspension. This demonstrated that the cancer differentiation cell model was successfully constructed for this cell line.

For the NSCLC cell line H2228, the growth curves demonstrated that the cell samples after 10μM and 20μM BrdU treatment also exhibited a reduced cell proliferation (Fig. 2F). Cells in culture with and without BrdU were harvested; RNA was isolated and tested for expression analysis. In the anchorage-independent growth test, there was no statistical significant difference between the colonies of H2228 cells before and after BrdU modification. However, the upregulation of cellular adhesion molecules like CX26, Laminin alpha 3 and EVA1 suggested that BrdU treatment may have also induced differentiation in this cell line (Fig.3 B, C and D).

Analysis of gene expression in the H526 lung cancer differentiation model revealed genes involved in cancer differentiation

Altered gene expression in parallel with the morphological changes were observed in BrdU induced differentiation of melanoma, neuroblastoma and small cell lung cancer cell lines [22,23,26]. We could show that the *in vitro* exposure of the non-adherent SCLC cell line H526 to BrdU resulting in morphological and functional changes was accompanied with the upregulation of three categories of genes potentially contributing to the cancer differentiation, i.e. growth factors (IGFBP-7), homeodomain transcription factors (PITX1, LAGY, HoxB2) as well as extra- and intra-cellular adhesion molecules (CX26, Laminin alpha 3 and EVA1). Representative images of the expression analysis are shown in Fig. 3.

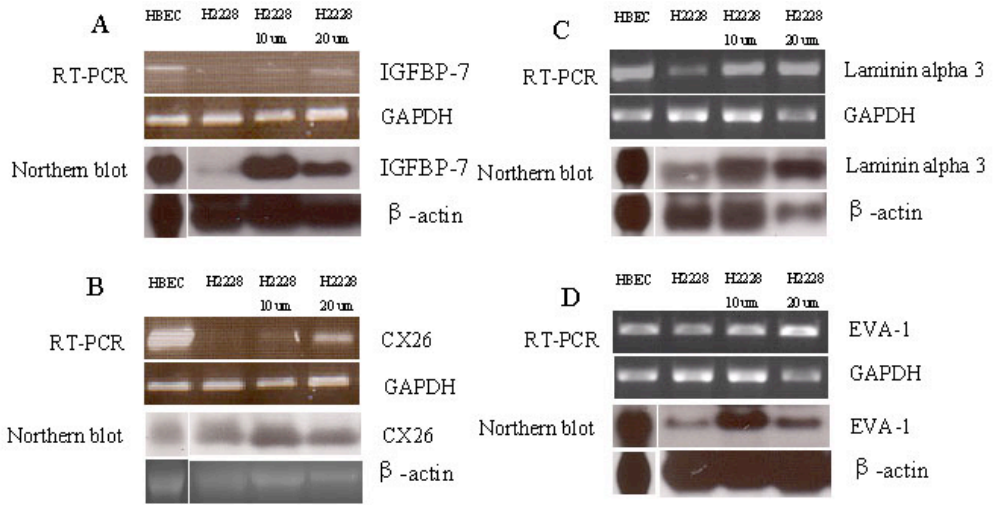

Fig. 3 Gene expression comparison in H2228 cell line between before and after BrdU treatment. The mRNA expression of IGFBP-7 (A) and Cx26 (B) was restored in BrdU-treated H2228 cells being particularly evident in the Northern blot analysis; Laminin alpha 3 (C) and EVA1 (D) were remarkably up-regulated in the H228 cells after BrdU treatment at mRNA level both by RT-PCR and Northern blot.

We selected these genes as targets of the expression analysis based on three reasons. First, all of them have been identified in our SSH libraries that were constructed by the comparison of lung cancer cell lines with normal human bronchial epithelial cells, and were down-regulated in a variety of lung cancer cell lines. Moreover, we reported that the low expression of IGFBP-7, Cx26, LAGY and PITX1 was linked to high grade, i.e. less differentiated tumors

[10-12,16,17,20]. Secondly, these three categories of genes importantly contribute to lung organogenesis [24,27] and have recently become the hotspots of differentiation and cancer research, e.g. the homeobox gene BARX2 that regulates chondrogenesis during limb development induces cadherin 6 expression and is a functional suppressor of ovarian cancer progression [28,29]. Furthermore, our lab has investigated several genes of these classes in carcinogenesis [12,20].

Homeobox genes constitute a superfamily of transcription factors that control cell differentiation and morphogenesis during embryonic development. These genes are characterized by the presence of a signature DNA sequence that encodes a 61 amino acid domain known as the homeodomain [30]. It is widely accepted that tumor development is an aberrant form of organogenesis. Homeobox genes represent classical examples of the intimate relationship between embryogenesis and neoplasia [47]. Aberrant expression of homeobox genes and a role in tumor differentiation and progression have been documented in a variety of solid tumors. For instance, Wt-Prox1 homeobox gene suppressed tumor cell proliferation *in vitro* and *in vivo*, and the mutant form of Prox1 lost its function; Prox1 negatively regulates tumor progression in hepatocellular carcinoma [31,32]. CDX-2 plays a vital role in the development and differentiation of intestinal epithelial cells. Of diagnostic importance is the loss of CDX-2 immunoreactivity in poorly differentiated colorectal ADCs [33]. TTF-1 mRNA and protein expression decreased significantly in adenomas and adenocarcinomas compared to normal lung tissue [34]. Positive TTF-1 staining strongly and independently correlates with survival of patients with primary adenocarcinoma of the lung [35].

Earlier, we reported the downregulation of three homeobox genes PITX1, LAGY and HoxB2 in a panel of lung cancerous cell lines relative to normal cells and the low expression of LAGY was linked to high grade tumors [10,11]. Recently, we found that in primary lung carcinomas, poorly differentiated tumors showed relatively low expression of PITX1 which was statistically related to high tumor stage. These data suggested the function of PITX1 in lung cancer differentiation and progression [16]. Specifically in the differentiation experimental model, PITX 1 expression was remarkably up-regulated in well-differentiated epithelial phenotype H526B by RT-PCR and Western blot analysis (Fig.4 E). The protein expression of HoxB2 and LAGY were up-regulated in BrdU-induced H526B cells compared

to the original SCLC cell line H526 (Fig.4 C and D); The reduction or loss of expression of three homeobox genes in lung cancerous cell line H526 was well restored in BrdU-derived epithelial-differentiation cell line H526B. All these data strongly suggested that homeobox genes played a crucial role in cancer differentiation processes, which was well consistent with our previous description in this part.

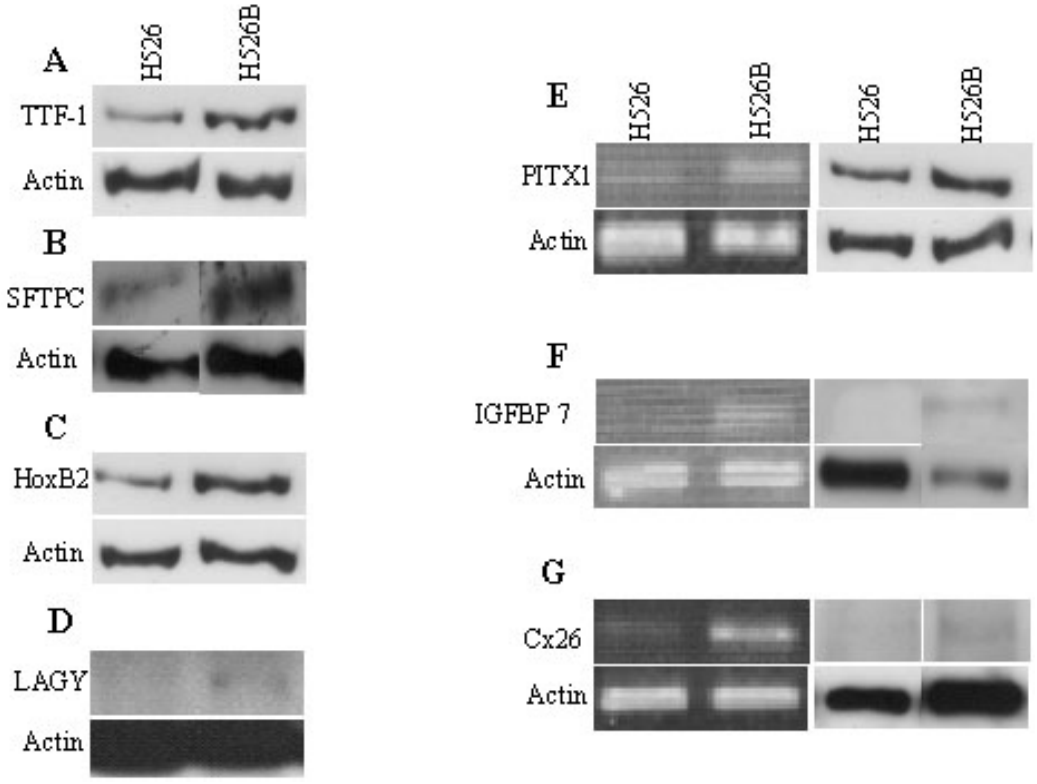

Fig. 4 Gene expression analysis of H526 and H526B cells. After BrdU modification, an increased protein expression was found for TTF-1 (A), SFTPC (B) and HoxB2 (C). An increased mRNA expression was evident for LAGY (D). Similarly the genes PITX1 (E), IGFBP-7 (F) and Cx 26 (G) were differentially expressed both on the mRNA (left) and protein level (right).

Paired-like homeodomain transcription factor 1 (PITX1), also known as Backfoot (BFT) and pituitary homeobox 1, was first isolated on the basis of its ability to induce expression of pituitary-specific genes being involved in organ development and left-right asymmetry. PITX1 protein acts as a transcriptional regulator and is detectable through the differentiation process of pituitary cells [36]. Many publications supported the role of PITX1 in human

carcinogenesis. RNA interference libraries targeting large proportions of the human genome uncovered that PITX1 was a novel suppressor gene that promotes the expression of a negative regulator of Ras [37]. PITX1 expression was also decreased in prostate and bladder cancers relative to their normal controls, furthermore, transduction of primary-derived human prostate RWPE-1 cells with the PITX1 gene induced very efficient anchorage-independent growth in soft agar assays, raising the possibility that PITX1 is likely a relevant tumor suppressor in human prostate cancer [38]. Together with our findings for this gene, PITX1 could vitally participate in the carcinogenesis and cancer differentiation.

Cell-cell and cell-matrix interactions are key events in growth control and the transdifferentiation of epithelial and mesenchymal cells. The disruption of the epithelial and mesenchymal homeostasis leads to changes in cell differentiation and proliferative potential. Epithelial-mesenchymal transition (EMT) can occur in physiological situations such as embryonic development or in pathological conditions such as cancer growth. In carcinogenesis, the loss of the epithelial phenotype starts from the loss of intercellular junctions during the multiple EMT processes [39]. Connexins (Cxs) are a family of membrane proteins constituting gap junctions which facilitate intercellular communication. Connexin 26 (Cx26) is a reliable early indicator of airway epithelial development and differentiations [40]. We previously demonstrated that an association between Cx26 protein expression and tumor grade [12]. In our differentiation model, the upregulation of Cx26 mRNA and protein expression after BrdU modification further underscores a role of this gene in cancer differentiation (Fig.4G).

Beside PITX1 and Cx26, the expression of IGFBP-7 was increased at mRNA and protein levels in BrdU-derived H526B and BrdU-treated H2228 cell lines compared to original ones (Fig.3A; Fig.4F). The potential role of IGFBP-7 in cancer differentiation was elucidated in this experimental system. In fact, regarding to the contribution of IGFBP-7 to differentiation, Walker reported that IGFBP-7-induced neuroendocrine (NE) differentiation and interacted with the novel protein 25.1 in lung cancer [41].

Expression and regulation of IGFBP-7 in colon and lung cancer and its potential role in cancer differentiation

Insulin-like growth factor binding protein 7 (IGFBP-7) is a member of IGFBP superfamily that itself belongs to the Insulin growth factor (IGF) family. The IGF signaling pathway plays a key role in regulating proliferation, differentiation, and apoptosis in mammalian organisms [42].

Originally, we firstly observed that IGFBP-7 is downregulated in human colon carcinomas similar to lung cancer and other cancer types which was in contrast to previous studies reporting an upregulation [43,44]. These findings induced our interest to study the mechanisms of IGFBP-7 regulation. A high level of aberrant DNA methylation was detectable in three colon cancer cell lines and three lung cancer cell lines devoid of IGFBP-7 expression while only sporadic methylation was seen in normal epithelial cells. Aberrant DNA methylation was the prominent determinant for the downregulation of IGFBP-7 in colon cancer and lung cancers [17,20]

In the expression analysis, IGFBP-7 not only showed a downregulation in a variety of colorectal and pulmonary cancer cell lines. By immunohistochemical analysis of tissue microarrays we furthermore found that the expression of IGFBP-7 protein differed statistically between colon carcinomas with different primary tumor stage and lymph node status. There was also a significant correlation between low expression of IGFBP-7 and high tumor grade tumors [17]. Similarly, lung tumors with higher grade exhibited lower IGFBP-7 protein expression [20]. In lung cancer we extended the analysis to functional studies. IGFBP-7 positive transfectants remarkably reduced the ability of colony formation in soft agar, suppressed the tumor growth rate in nude mice and increased the number of apoptotic cells [20]. Thus, our expression analysis and the *in vivo* and *in vitro* experiments suggested that IGFBP-7 plays a role in colon and lung carcinogenesis as a tumor suppressor being consistent with published data in prostate and breast cancer [45,46]

In our cancer differentiation model, IGFBP-7 expression was restored in the BrdU-derived cell line H526B (Fig.4F). In the BrdU-treated H2228 cell line, the mRNA expression of IGFBP-7 was increased by Northern blot and RT-PCR (Fig.3A). All these findings lend further support to the hypothesis that IGFBP-7 has a significant role in the carcinogenesis as a

tumor suppressor by inducing differentiation of the cancer cells.

It is well known that DNA methylation is indispensable for embryonic development. Aberrant expression of differentiation regulatory genes may drive cells back to an undifferentiated, proliferation-promoting status and predisposes these cells for neoplastic transformation [27,47]. Some potential differentiation regulatory genes have been reported to be downregulated in several cancer types for epigenetic mechanisms (Table 2).

Table 2 Examples of aberrantly expressed homeobox genes in solid tumors

Mechanism of downregulation	Genes	Expressionn pattern	Cancer types
Aberrant DNA methylation	*CDX2*	Expressed during normal gut development and in adulthood. Loss of expression in colorectal cancer correlated with promoter methylaiton.	Colorectal cancer
Aberrant DNA methylation	*HOXA5*	Loss of expression occurs in >60% breast cancers and correlates with promoter methlation.	Breast cancer
Aberrant DNA methylation	*ALX3*	High expressed in normal brain. Loss of expression in neuroblastomas correlates with promoter methylation.	Neuroblastomas

∗ Reviewed in reference Samuel and Naora 2005

Induction of differentiation has become a fascinating issue in cancer therapy because it might be potentially less toxic than conventional approaches. For instance, patients with dedifferentiated thyroid cancer (DTC) or acute promyelocytic leukaemia (APL) treated with all-trans-retinoic acid (ATRA) showed an effect on the differentiation status of DTC while the APL patients showed complete remission rates in 90-95% of the cases [48,49].

In summary, we established an *in vitro* differentiation model by the treatment of lung cancer cells with BrdU. Morphological changes indicative for cancer differentiation were detectable and several classes of differentiation associated genes were upregulated. Among these, particularly IGFBP-7 was investigated in both lung and colorectal cancers and shown to be a tumor suppressor being downregulated by epigenetic silencing via methylation. Identification and characterization of genes involved in cancer differentiation should contribute to the development of new anticancer therapies.

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Erklärung

„Ich, [Fei, Ye], erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: [.Characterization of genes involved in cancer differentiation.] selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe."

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