Cell. Mol. Life Sci. 65 (2008) 591 – 604
1420-682X/08/040591-14
DOI 10.1007/s00018-007-7392-4

Cellular and Molecular Life Sciences

Review

The Helicase-Like Transcription Factor and its implication in cancer progression

G. Debauve[a], A. Capouillez[a,b], A. Belayew[a,*] and S. Saussez[b]

[a] Laboratory of Molecular Biology, Faculty of Medicine and Pharmacy, University of Mons-Hainaut, Pentagone 3A- Avenue du Champ de Mars, 6, B-7000 Mons (Belgium), Fax: +32-65-373583, e-mail: alexandra.belayew@umh.ac.be
[b] Laboratory of Anatomy, Faculty of Medicine and Pharmacy, University of Mons-Hainaut, Mons (Belgium)

Received 24 August 2007; received after revision 17 October 2007; accepted 23 October 2007
Online First 24 November 2007

Abstract. The helicase-like transcription factor (HLTF) belongs to the SWI/SNF family of chromatin-remodeling factors. Several SWI/SNF genes are disrupted in cancer, suggesting their possible role as tumor suppressors. Similarly, the *HLTF* gene was found to be inactivated by hypermethylation in a significant number of colon, gastric and uterine tumors, indicating that *HLTF* silencing may confer a growth advantage and that *HLTF* could be considered as a tumor suppressor gene. However, 20-fold *HLTF* overexpression was detected in various transformed cell lines, suggesting that *HLTF* could be associated with neoplastic transformation and act more like an oncogene. Moreover, *HLTF* activation was recently linked to the initial steps of carcinogenesis in an experimental model of estrogen-induced kidney tumors. Those apparently contradictory observations suggest that HLTF might play various roles in cancer. In this review, we will try to reconcile all these data in order to specify the role of HLTF in cancer progression.

Keywords. Cancer, HLTF, oncogene, PAI-1, promoter methylation, SMARCA3, SWI/SNF, tumor suppressor.

Introduction

The DNA contained in a human cell would be 2 m long if stretched end-to-end. In order to fit into a 10-μm nucleus, this DNA has to be compacted about 200,000-fold. Eukaryotic cells have solved this packaging problem by folding their DNA into a chromatin structure where the negative charges of its sugar phosphate backbone are neutralized by the basic residues (Arg, Lys) of the histone proteins. The building block of chromatin is the nucleosome in which 147 bp of DNA are wrapped approximately two turns around an octameric complex composed of two molecules of each of the four canonical histones H2A, H2B, H3 and H4. Consecutive nucleosomes line up, generating a fiber with a diameter of 11 nm, termed beads-on-a-string, which can be further compacted into a 30-nm fiber through incorporation of a linker histone, H1. Nucleosomes are now recognized as dynamic participants in many chromosomal processes including transcription, replication, DNA repair, kinetochore and centromere construction and telomere maintenance [1]. Nucleosomes themselves are stable and show limited mobility, and their dynamic properties are due to the action of chromatin-modifying and -remodeling complexes. Modifying complexes add or remove chemical groups at particular residues on the histone proteins. The most studied modification is the addition of an acetyl group on the side chain of lysines by histone acetyl trans-

* Corresponding author

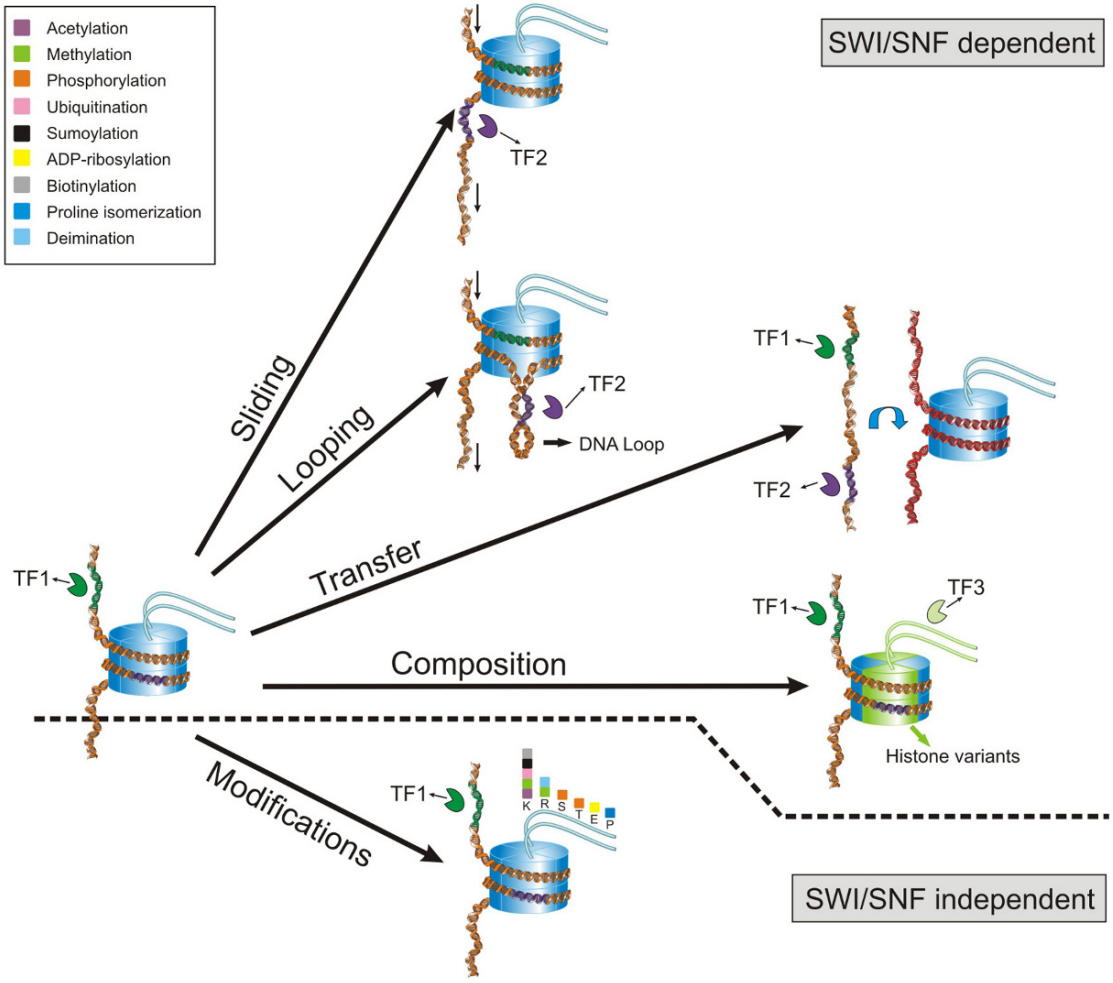

Figure 1. Dynamic properties of nucleosomes. Above the dashed line: ATP-dependent mechanisms. Sliding: nucleosome repositioning allows the binding of a transcription regulatory factor (TF) to its site on nucleosomal DNA (dark-blue segment) and/or inhibits the binding of another one (green segment). Remodelers are necessary to provide rapid access to nucleosomal DNA by sliding of the octamer along the DNA. Alternatively, the site might be accessed on the surface through the generation of a DNA loop (Looping) or through histone-octamer transfer to an acceptor DNA (Transfer). Composition: remodeling complexes can remove the canonical H2A-H2B dimers and replace them with histone variants (green), forming a variant nucleosome with unique tails that might bind a unique transcription regulatory factor (TF3). Below the dashed line: ATP-independent mechanism. Modifications: histone tail modifications result from various enzyme activities (see list) and are targeted by transcription regulatory factors that can influence chromatin dynamics and function.

ferases (HATs) that thus loosen ionic bonds between DNA and histones in the nucleosome. The histone deacetylases (HDACs) restore the positive charge on the lysine side chains and strong ionic bonds with the negative charges of the DNA phosphates, favoring the building of a compact chromatin structure. Most of the time, these modifications occur on the histone tails (the amino- and carboxyl-terminal domains) and are subsequently recognized by transcriptional regulators and other factors. Modifying complexes work in concert with chromatin-remodeling complexes, which restructure, mobilize and eject nucleosomes to regulate access to the DNA [2] (Fig. 1).

Chromatin helps eukaryotic cells to store DNA, but creates an impediment to DNA access during transcription, replication, repair and recombination. Therefore, modulation of chromatin structure plays an important role in the regulation of these nuclear processes in eukaryotes. The cell has developed dedicated modification mechanisms which locally alter chromatin structure to facilitate or repress DNA access. These mechanisms include histone covalent modifications, histone replacement by histone variants and ATP-dependent chromatin remodeling. The latter process is mediated by DNA-translocating motors that utilize the energy derived from ATP hydrolysis to perturb the histone-DNA interactions [3]. These remodelers can cause: (i) nucleosome sliding, in which the position of a nucleosome on the DNA changes, (ii) the creation of a remodeled state, in

which the DNA becomes more accessible but the histone remains bound, (iii) the complete dissociation of DNA and histones or (iv) histone replacement with a histone variant (Fig. 1).

Currently, four different classes of remodeling complexes are recognized: SWI/SNF, ISWI, Mi-2 and Ino80. The SWI/SNF (mating-type switching/sucrose non-fermenting) chromatin-remodeling complexes were initially discovered in yeast where their inactivation by mutation caused global disruption of transcription regulation. These chromatin-remodeling complexes were subsequently found in all eukaryotic cells, in which their activities can result in transient unwrapping of the end DNA from the histone octamer, causing DNA looping or nucleosome sliding to different translational positions (Fig. 1), [reviewed in ref. 3]. All of these modifications will change the accessibility of nucleosomal DNA to transcription factors [4]. Remodeling can also involve nucleosome loss or ejection as described for the SWI/SNF-related complex RSC, which is able to transfer histone octamers from donor nucleosomes to acceptor DNA [5]. The SWI/SNF complexes normally consist of 9–12 subunits, but only four of these – BRG1 or BRM, SNF5, BAF155 and BAF170 – are required *in vitro* to remodel nucleosomes at a rate that is comparable to that of the entire complex [6]. The central core subunits BRG1 and BRM contain an ATPase domain and a bromodomain that can specifically interact with acetylated lysines in histone tails or in other proteins. The SNF5 subunit (also known as INI1 or SMARCB1) is present in all known variants of the SWI/SNF complex and is specifically inactivated or mutated in atypical teratoid/malignant rhabdoid tumors (ATRTs and MRTs), highly aggressive cancers of early childhood [7, 8]. hSNF5 mutations were also found associated with some other neoplasms, including chronic myeloid leukemia, choroid plexus carcinoma, medulloblastoma and central primitive neurectodermal tumors [3]. Typically, the wild-type allele is either lost or mutated in the tumor, consistent with a classic tumor suppressor phenotype. Roberts et al. [9] utilized a reversibly inactivated conditional allele to study SNF5 function in mice and showed that it resulted in a highly penetrant and extremely short latency development of lymphomas and rhabdoid tumors.

The helicase-like transcription factor (HLTF), a member of the SWI/SNF family, was recently discovered to be involved in cancer progression in various ways. Several studies have revealed *HLTF* promoter hypermethylation in human colorectal [10–21], gastric [12, 22–25], oesophageal [12, 26] and uterine [27, 28] cancers suggesting that *HLTF* silencing may play a crucial role in cancer. Recently, HLTF expression was investigated in a hamster model of kidney tumors induced by diethylstilbestrol (DES). Interestingly, HLTF was detected very early (after 2 months of DES treatment) during the tumor progression in small pre-neoplastic buds [29]. These data provide evidence that *HLTF* gene activation is linked to the initial steps of carcinogenesis and should be investigated in early stages (dysplasias) of other human cancers. HLTF can thus act as either a positive or a negative regulatory factor in tumor development, depending on the histological type and stage of progression.

The discovery of HLTF

The HLTF protein (also called SMARCA3 in OMIM) can specifically interact with DNA target sequences located in different genes. This specific DNA-binding activity was independently used by different groups to screen cDNA expression libraries and to isolate the HLTF cDNA. These library screenings were performed with different target sequences derived from the following genes: (i) the HIV promoter and SV40 enhancers: HIP116 [30], (ii) the human or mouse plasminogen activator inhibitor 1 (PAI-1) promoter: HLTF [31]; P113 [32], (iii) the rabbit uteroglobin promoter: RUSH [33], (iv) the myosin light chain gene enhancer: Zbu1 [34] and (v) a *cis* element in the β-globin locus control region (LCR): HLTF [35]. However, direct evidence for a role of HLTF in transcription has only been demonstrated for the *PAI-1* gene [31, 32, 36] and for the β-globin LCR, where HLTF is involved in the terminal differentiation corresponding to adult gene activation [35]. In fact, Mahajan and Weissman [35] have shown that HLTF overexpression in K562 cells does not affect the endogenous levels of γ- and ε-globin mRNAs but markedly activates *β-globin* transcription.

Regulation of HLTF expression

No data are available on the regulation of human *HLTF* gene expression. Cloning and characterization of the rabbit *HLTF (RUSH)* promoter have established that it does not have a TATAA motif in the vicinity of the transcription start site [37]. A unique initiator/downstream promoter element (Inr-DPE) combination directs accurate initiation of *RUSH* transcription. The highly conserved nature of the Inr-DPE cassette in the human and rabbit *HLTF* genes suggests that some aspects of transcriptional activation are also conserved. *RUSH* is negatively regulated by two Sp1 sites (also highly conserved in human *HLTF*) by NF-Y and RUSH itself [37].

In the uterus, rabbit *HLTF (RUSH)* is regulated with steroids at the level of transcription and mRNA

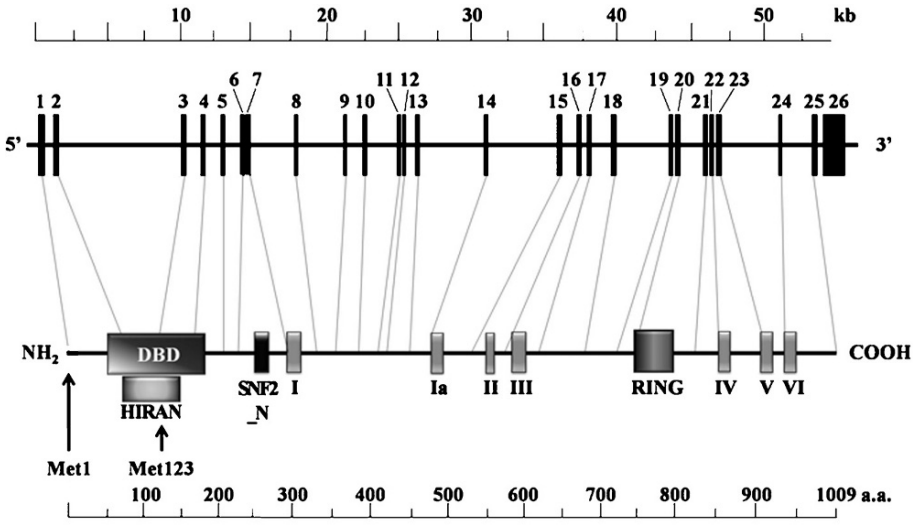

Figure 2. The intron/exon structure of the human *HLTF* gene (GenBank no. AJ418064) is given above the domain organization of the encoded protein. The 5' end of each exon is joined to the NH_2 end of the protein region it encodes. Vertical arrows indicate the alternative translation initiator sites (Met1 and Met123). The DNA-binding domain (DBD), the HIP116-RAD5 N-terminal domain (HIRAN [42]), the SNF2 family NH_2-terminal domain, and the seven helicase domains (numbered from I to VI) are represented by rectangles. The RING domain is represented by a square.

splicing [33]. Two spliced variants have been characterized: RUSH-1α is the progesterone-dependent splice variant and RUSH-1β is the estrogen-dependent splice variant [33]. RUSH proteins have been described to play a role in *uteroglobin* gene regulation in cooperation with progesterone and prolactin [38]. In general, prolactin signal transduction is mediated by the sequence-selective binding of Stat5a, which is induced by hormone receptor binding [39]. However, in the absence of Stat-binding sites on the *uteroglobin* promoter, a RUSH-binding site (–126/–121) mediates the ability of prolactin to augment progesterone-dependent transcriptional activation of *uteroglobin* [40].

The HLTF gene and encoded proteins

The *HLTF* gene is located on chromosome 3q25.1-q26.1 [41]. It is 56.4 kb long and is composed of 26 exons. Two *HLTF* mRNAs (5.4 and 4.5 kb) are expressed in most human tissues and result from the alternative splicing of intron 25 located outside of the coding region in the 3′ untranslated region (3′UTR) [31]. Two proteins (HLTFMet1 and HLTFMet123) encoded by the same reading frame but differing in translation start site (Met1 or Met123 codons) are produced from each of the two mRNAs [31]. Only the shorter variant, HLTFMet123, exhibits transcriptional activity in synergy with the Sp1 or Sp3 transcription factor to which it binds through its last 80 residues [36]. HLTF has a RING finger motif, a SNF2 family amino-terminal domain as well as the seven DNA helicase domains characterizing the SWI/SNF family (Fig. 2). Although no helicase activity has been demonstrated, it has been shown that native HLTF possesses double-strand DNA-dependent ATPase activity *in vitro* and that the ATPase activity is preferentially stimulated by addition of its DNA-binding site [30]. Thus, HLTF might function as a site-specific ATPase to modify the local chromatin structure, thereby facilitating binding of other transcription factors (Fig. 1).

The HLTF DNA-binding domain (DBD) was initially described in the amino-terminal part of the protein (a.a. 38–180) [30, 31]. Recently, Iyer et al. [42] described a shorter version of this domain (a.a. 60–154), conserved in several proteins, that they named HIRAN for HIP116 and RAD5 N-terminal domain. Using computational analysis, they showed that the HIRAN domain along with some other DBDs potentially play a major role in recruiting repair and remodeling enzymes to specific sites on DNA, and might thereby have a role in certain cell cycle checkpoints arising from replication fork stalling and post-replication damage [42].

The SNF2 family amino-terminal domain, located between the DBD and the first helicase domain (Fig. 2), is present in proteins involved in a variety of processes including transcription regulation (e.g. SNF2, STH1, brahma, MOT1), DNA repair (e.g. ERCC6, RAD5, RAD16), DNA recombination (e.g. RAD54) and chromatin unwinding (e.g. ISWI), as well as in a number of other proteins for which we have little functional information (e.g. lodestar, ETL1) [43, 44]. Flaus et al. [45] have aligned all the known protein sequences of the helicase-related regions to update the classification of Snf2 family members. Based on their similarity, HLTF, RAD5 and RAD16 were regrouped in the same subfamily. Paradoxically, although RAD5 and RAD16 are both involved in DNA repair pathways [46–49], no link with DNA repair has been reported for HLTF.

The C3HC4 motif or RING finger was found close to the carboxyl terminus of HLTF, inserted between two

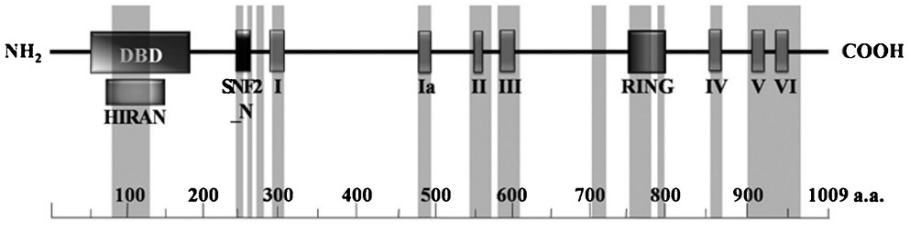

Figure 3. Schematic representation of the conserved HLTF domains. Multiple sequence comparison by log expectation (Muscle, EMBL-EBI) was performed on HLTF and HLTF putative orthologous sequences in order to identify the most conserved domains. All the sequences came from the Ensembl ortholog software (release 43, http://www.ensembl.org). The species considered were: *Homo sapiens* (human), *Mus musculus* (mouse), *Oryctolagus cuniculus* (rabbit), *Pan troglodites* (chimpanzee), *Canis familiaris* (dog), *Bos taurus* (cow), *Ratus norvegicus* (rat), *Saccaromyces cerevisiae* (yeast), *xenopus laevis* (African clawed frog), *gallus gallus* (chicken), *Danio rerio* (zebrafish), *Tetraodon nigroviridis* (green spotted pufferfish). The most conserved regions are indicated by pale-grey columns.

ATPase motifs, in an arrangement similar to that observed in the yeast RAD5 protein (Fig. 2). A RING finger domain contains a C3HC4 motif that binds to two zinc atoms in a cross-braced system, where the first and third pairs of cysteine/histidine form the first binding site and the second and fourth pairs form the other. Interestingly, the HLTF RING domain shares 42% sequence identity with the BRCA1 RING and 57% with its yeast homolog, RAD5B. A role in ubiquitination that targets proteins for degradation by the proteasome was demonstrated for those two proteins [50–52] but has not been investigated for HLTF.

Chilton and colleagues showed that the rabbit HLTF (RUSH) RING domain has also been highly conserved throughout evolution [53]. They demonstrated that the RUSH RING domain interacted with the RING-finger-binding protein (RFBP), an atypical type IV P-type ATPase that could play a role in the subnuclear trafficking of transcription factors with RING motifs [54].

In a similar approach, the HLTFMet1 sequence was searched against the NCBI databases and analyzed using the BlastP program. Significant homology was seen between HLTF and a number of proteins from different species, including SMARCA3 (chimpanzee, 99% identity; dog, 93% identity; cow, 91% identity), RUSH-1α and β (rabbit, 91% and 90% identity, respectively), P113 (mouse and rat, 83% identity), MGC131155 (African clawed frog, 63% identity) and RAD5B (yeast, 25.7% identity). A multiple alignment between human HLTF and its known or putative orthologs identified conserved regions and showed that the HLTF domains described above have been highly conserved through evolution (Fig. 3). An additional domain (coordinates 700–720) is highly conserved but has no identified function.

Developmental regulation

Gong et al. [34] isolated *HLTF* under the name *Zbu1* and studied its expression during mouse embryogenesis. They demonstrated that *HLTF* mRNAs accumulate quite late (17–18 days post-coitum) and predominate in fetal skeletal muscle, heart and brain. In contrast to the late mRNA accumulation, their immunohistochemical analysis revealed a relatively high protein level exclusively in the embryonic heart at 11.5 days, followed by a gradual increase in skeletal muscle [34]. In fact, the protein accumulation was asynchronous among the tissues which initially expressed the gene, starting with the embryonic heart and only subsequently accumulating in skeletal muscle. The significance of this expression pattern is not clear; nevertheless, the high activity of the gene predominantly in adult post-mitotic tissues – skeletal muscle, heart, brain, lens and mature sperm – suggests a role in the maintenance of the differentiated state [34].

DNA hypermethylation and cancer

Cancer is characterized by the presence of six essential alterations in cell physiology that dictate malignant growth: self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. Each of these capabilities newly acquired during tumor development represents the successful breaching of an anticancer defense mechanism [55]. In recent years, additional mechanisms affecting gene expression have been implicated in tumorigenesis. Two major areas of epigenetic alterations – DNA methylation and histone modifications – are known to have profound effects on controlling gene expression [56].

DNA methylation occurs almost exclusively on a cytosine in a CpG dinucleotide and is achieved by

adding a methyl group to position 5 of a cytosine ring mediated by DNA methyltransferases (DNMTs) [57]. In normal human DNA, 3–6% of all cytosines are methylated and the potentially 'methylable' CpG dinucleotides are not randomly distributed in the human genome [58]. The CpG-rich regions known as CpG islands, which span a few hundred base pairs in the 5′ region (promoter, 5′UTR and exon 1) of many genes, are usually unmethylated in normal cells, whereas the sporadic CpG sites in the genome are normally methylated [59]. There is a gradual reversal of this pattern during aging that leads to sporadic methylation in the CpG islands and a global loss of methylation, but this change is particularly pronounced during carcinogenesis (Fig. 4).

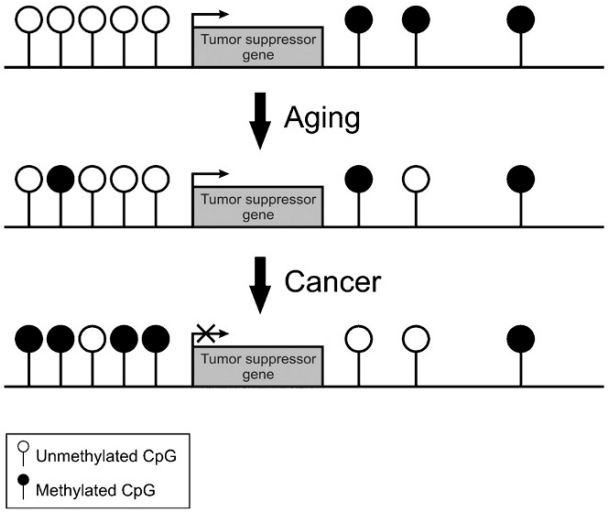

Figure 4. CpG islands normally remain unmethylated (white circles), whereas the sporadic CpG sites located in the rest of the genome are often methylated (black circles). With aging, there is a gradual reversal of this phenomenon. During carcinogenesis, this change is much more dramatic, leading to a global genome hypomethylation associated with hypermethylation of CpG islands. The results are chromosomal instability and silencing of some important tumor-suppressor genes [adapted from ref. 56].

Methylation of CpG islands in promoter regions is often associated with gene silencing, and aberrant DNA methylation occurs in most cancers, leading to the silencing of some tumor suppressor genes [56, 60]. Two basic mechanisms have been proposed for this process: first, DNA methylation can directly repress transcription by blocking transcriptional activators from binding to cognate DNA sequences, and second, methyl-CpG-binding proteins (MBPs) recognize methylated DNA and recruit corepressors such as HDACs to directly silence gene expression [57].

HLTF promoter hypermethylation in colorectal cancer

HLTF in colon tumors

There is now strong evidence that a series of genetic alterations affecting both some oncogenes and some tumor suppressor genes are involved in the pathogenesis of human colorectal cancer. Specific gene mutations initiate the formation of adenomas (e.g. *APC* mutations) and others drive the malignant transformation of the adenomas (e.g. *TP53* mutations) in a multistep progression model of colon cancer formation [61]. More recently, epigenetic alterations have also been found to occur commonly in colon cancer. However, although methylated genes are commonly detected in colon neoplasms, it is still not clear whether DNA methylation is a primary pathogenic event or a secondary phenomenon. Another area of controversy related to aberrant DNA methylation is the concept developed by Toyota and Issa [62], in which certain colon cancers are typified by a high frequency of gene promoter methylation and represent a distinct group termed the CpG island methylator phenotype (CIMP+). Tumors exhibiting this CIMP+ phenotype show concordant CpG island methylation affecting multiple genes, including *hMLH1*, *P16* and *THBS1.* Recent studies have even demonstrated that one subset of CIMP+ colon cancers presented unique clinicopathological and molecular features (e.g. BRAF V600E mutations) [63, 64]. However, the timing and the role of CIMP+ in the polyp cancer progression sequence has not yet been characterized clearly.

HLTF promoter methylation was initially reported in colon cancer by Moinova et al. [20]. In their report, the authors examined the genomic sequence upstream of the *HLTF* gene (GenBank accession no. NT_005616), where they identified a putative promoter containing a CpG-dense region that could be methylated (Fig. 5). All colon cancer cell lines that lacked *HLTF* gene expression (9/34 colon cancer cell lines) demonstrated methylation of CpG sites within the putative promoter, while methylation was not detected in the HLTF-expressing cell lines. In tumors, *HLTF* gene methylation was detected in 27 of 63 primary colon cancers (43%). In addition, Hibi et al. [12] demonstrated that *HLTF* methylation was detectable in 25 out of 76 primary colon cancers, suggesting that *HLTF* was a common target for methylation. Recently, the same research team showed that colorectal cancers without lymph node metastases were highly methylated (colorectal cancers with more than three methylated genes including *HLTF* corresponded to this highly methylated group, HMG) and presented a better prognosis [13]. Hibi and Nakao [14] have also

demonstrated that highly methylated colorectal cancers were significantly correlated with a poorly differentiated histology, including mucinous adenocarcinoma.

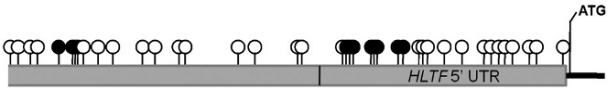

Figure 5. *HLTF* promoter methylation. Putative *HLTF* promoter CpG sites are shown with circles. Shaded circles represent CpG sites that were tested in MS-PCR assays [adapted from ref. 20].

Moinova et al. [20] showed that *HLTF* methylation was strongly correlated with CIMP+ and with *hMLH1* gene methylation. However, *HLTF* methylation was also observed in 22 % of CIMP– tumors [20]. It is thus unclear whether this represents two different subsets of tumors with potentially different underlying predispositions for aberrant methylation of the *HLTF* locus.

The high frequency of *HLTF* methylation observed in colon cancer suggested that the silencing of this gene could confer a selective advantage. Considering this hypothesis, Moinova et al. [20] transfected three different HLTF-deficient cell lines with an HLTF expression vector and observed 75 % suppression of colony growth in soft agar. The results of these experiments suggest that *HLTF* silencing may confer a growth advantage on some colon cancers, and hence that *HLTF* could be considered a colon cancer tumor suppressor gene [20].

The frequency of *HLTF* promoter methylation increases drastically between normal colonic tissues and adenomas (Fig. 6), but there is no difference between adenomas, primary cancers and liver metastases [16]. Bariol et al. [65] failed to show any significant difference in the frequency of methylation markers among small adenoma, large adenoma and cancer, whereas Rashid et al. [66] reported that methylation is more frequent in adenomas with tubulovillous or villous histology. These findings provide further correlative evidence that *HLTF* silencing might drive the initiation and the progression of colon cancer formation. Moreover, *HLTF* methylation could be useful as a diagnostic biomarker, as it can distinguish normal tissue from colonic neoplasm or differentiated carcinomas from poorly differentiated carcinomas.

Detection of the hypermethylated *HLTF* gene in patients with colorectal cancers:

Methylated DNA derived from primary colorectal cancers can be detected not only in the tumor tissue itself but also in serum [67] and stool samples of these patients [68]. In order to detect small quantities of

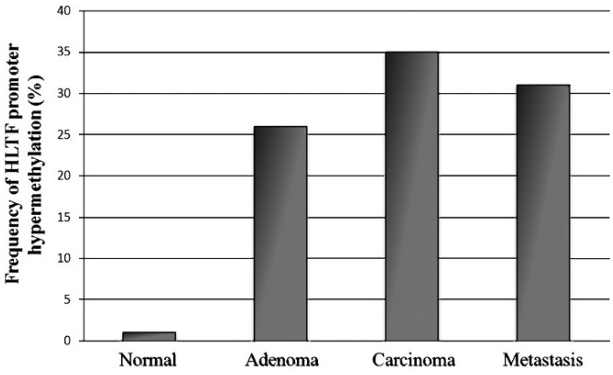

Figure 6. *HLTF* methylation frequency in liver metastases, colorectal cancers, adenomas and normal colonic tissues. Methylation was more frequently detected in metastasis, colorectal cancer and adenoma (all $p < 0.001$) than in normal tissues [adapted from ref. 16].

methylated DNA, a methylation-specific PCR (MSP) was developed [69] using a sodium bisulfite treatment that converts unmethylated cytosines to uracils prior to PCR. Subsequent PCR amplification was performed using primers designed to distinguish methylated from unmethylated DNA, taking advantage of the sequence differences resulting from bisulfite modification. MSP is rapid and sensitive to 0.1 % methylated alleles of a given CpG island locus. The precise mechanism by which DNA is released into the bloodstream still remains uncertain. Malignant cell death results mainly from necrosis, autophagy and mitotic catastrophe. These events are associated with a high DNA integrity compared with apoptosis, which involves programmed enzymatic digestion of DNA. Highly proliferative, aggressive tumors release tumor DNA into the intercellular space that is then drained by lymphatics into the bloodstream [70]. Thus, circulating tumor DNA is a representative marker for active, fast-growing tumors with intense neovascularization and dense lymphovascular invasion [70]. Wallner et al. [71] identified *HLTF* as a promising methylation marker in the serum of patients with colorectal cancers because it was not methylated in the serum of healthy controls and was methylated more frequently in metastatic than in local disease. The detection of *HLTF* methylation was significantly associated with tumor size, tumor stage and poor prognosis [71]. On the basis of these results, the authors proposed that the determination of DNA methylation has the potential to become an independent pre-therapeutic predictor of recurrence that could be used to identify patients at risk and who could benefit from adjuvant chemotherapy [71].

Recently, several investigators have demonstrated the feasibility of detecting epigenetic changes in DNA from the stools of patients with colorectal cancer [72–

74] or benign polyps [75]. In contrast to blood, altered DNA arises from the neoplasm rather than from the bloodstream and is released into the lumen continuously via exfoliation rather than intermittently via bleeding. Leung et al. [17, 19] have studied the methylation status of six tumor-related genes (among which *HLTF*) in fecal samples in order to find ways to overcome the low sensitivity of a single methylation marker. Methylation was not detected in stool samples from healthy patients, whereas the mean number of methylated genes in the fecal DNA of cancer patients was 1.1 (range, 0–3) [17]. Concordant methylation patterns were detected in the tumor and the paired fecal DNA but the presence of methylation in fecal DNA was not associated with any clinicopathological characteristics of the tumor. Moreover, the frequency of *HLTF* methylation was comparable in the stool samples of patients with colorectal cancer and adenoma (20%) [19].

HLTF promoter hypermethylation in gastric carcinoma

Apart from allelic loss and somatic mutations, epigenetic alterations are increasingly recognized to play a pivotal role in gastric carcinogenesis [76]. DNA methylation of tumor-related genes has been shown to occur in early stages of stomach carcinogenesis and increases in parallel with cancer evolution [77, 78]. Although several genes have been implicated in tumor progression and prognosis [79], some studies have indicated that methylation of a single gene has little or no prognostic significance [80]. In contrast, methylation of multiple genes was linked to poor prognosis [25]. In addition, the frequency of *TP53* mutations in gastric cancer decreased in parallel with the increase in the number of methylated genes [25]. Gastric cancers feature the CpG island methylator phenotype and these CIMP+ gastric cancers tend to present DNA methylation of the *P16*, *hMLH1* and *RIZ1* genes, suggesting that the CIMP status corresponds to a succession of important events involved in gastric carcinogenesis [81–83].

The aberrant methylation of the CpG island at the promoter region of the *HLTF* gene has been detected in approximately 20–55% of primary gastric cancers, suggesting that *HLTF* methylation is a frequently encountered feature of these carcinogenic processes [12, 22–25]. As in colorectal cancers, *HLTF* promoter methylation has been observed frequently in CIMP+ gastric cancers [25]. This promoter methylation tends to be more frequent in patients with family histories of gastric cancer than in those without [23]. Moreover, for patients with family histories, *HLTF* methylation occurs with significantly different rates of prevalence in the early (70–90%) and advanced (15–40%) stages. These results indicate that *HLTF* silencing plays a role in the early phases of gastric carcinogenesis in patients with family histories of the disease. *HLTF* mRNA expression has been studied in different gastric carcinoma cell lines and Hamai et al. [22] have shown that the KATO-III cells present loss of *HLTF* expression associated with its promoter methylation. This loss was rectified by treatment with either 5-aza-2′-deoxycytidine (5AZA), or a demethylating agent, and trichostatin A (TSA), an histone HDAC inhibitor. A chromatin immunoprecipitation assay revealed that the acetylation levels of histones H3 and H4 in the 5′ CpG island of the *HLTF* gene were inversely associated with DNA methylation status. These findings support a model in which methyl-CpG-binding domain proteins act as anchors on methylated DNA, recruiting accessory proteins, such as HDAC, that can modulate chromatin structure and transcriptional activity of the gene [22].

HLTF promoter hypermethylation in esophageal squamous cell carcinoma

Esophageal squamous cell carcinoma (ESCC) is a particularly aggressive cancer with a poor prognosis. Several studies have indicated that a series of genetic changes in dominant oncogenes (*cyclin D1* and *hst1/int2*) and tumor suppressor genes (*TP53* and *p16*) are involved in the pathogenesis of human ESCC [84–87]. The *p16* gene was found to harbor promoter hypermethylation associated with the loss of protein expression in 82% of the ESCCs studied, making this gene silencing a high malignancy factor [88]. Concerning *HLTF*, methylation was detected in only 1 ESCC out of 40 (3%), suggesting that it is not a common target for epigenetic gene silencing in ESCC [12].

HLTF promoter hypermethylation in uterine cancer

Epidemiological and laboratory data suggest that the presence of specific human papilloma virus (HPV) subtypes and the integration of their DNA into the cervical cell genome are important factors in the development of cervical neoplasia [89]. However, since the majority of patients with HPV infection do not develop invasive lesions, HPV infection alone is probably insufficient for complete neoplastic transformation of cervical cells, implying the possible involvement of other genetic and epigenetic events that have not yet been elucidated. Dong et al. [90] analyzed the methylation pattern of the promoter regions of six tumor-related genes in cervical carcinoma and found that 79% of cervical tumors showed promoter hypermethylation at one or more genes.

Kang et al. [27] have studied the *HLTF* methylation profile in different histologic types of uterine cancers.

Previous studies suggested that the hypermethylation pattern was dependent on histologic type. For example, promoter hypermethylation of the *p16* and *DAPK* genes was detected more frequently in cervical squamous cell carcinomas (CSCCs) than in cervical adenocarcinomas (CAs), pointing to a distinct pathway of progression for these different tumor types [90]. *HLTF* is globally hypermethylated in 22% of uterine cancers but was more frequently methylated in CA (43%) and in endometrial adenocarcinoma (EA, 48%) than in SCC (3%) [27]. These findings indicate that *HLTF* promoter hypermethylation may predispose tissues to the development of specific types of human uterine cancer.

HLTF overexpression and carcinogenesis

HLTF expression has been studied both in normal tissues and in established cell lines [34]. It has been shown that *HLTF* is ubiquitously expressed in normal tissue and that its mRNAs are more abundant in skeletal muscle, heart and brain (see above). Northern blot analysis has also revealed that *HLTF* transcript levels are higher by ~20-fold in a variety of transformed cell lines compared to non-transformed human fibroblast cells or human heart tissue [34]. Gong et al. have also demonstrated that *HLTF* induction is associated with loss of *TP53* heterozygosity in Li-Fraumeni fibroblasts, which suggests a link between *HLTF* expression and oncogenic transformation [34]. Nevertheless, the role of HLTF in the progression toward a transformed phenotype is not clear, since no variation in *HLTF* mRNA accumulation was observed in fibroblasts from a *TP53*-null mouse, even after whole-body γ-irradiation [34].

In a recent study, we investigated HLTF protein expression using immunohistochemistry in a hamster model of estrogen induced kidney tumors [29]. A strong labeling was detected in small tumor buds, making HLTF an early cancer marker in this model. Although every cell was stained for HLTF at this early stage, the number of HLTF-positive cells decreased to 10% with cancer progression, and these positive cells were dispersed in the tumor mass. These data led to the conclusion that *HLTF* gene activation could be linked to initial steps of carcinogenesis in this model and should be investigated in early stages of other neoplasms.

HLTF and PAI-1

Blood clots can be dissolved at a site of injury when the structural integrity of damaged areas is restored. This process involves the proteolysis of the clot fibrin by plasmin, a serine protease formed by proteolytic activation of plasminogen, by either of two activators, urokinase-type plasminogen activator (uPA) or tissue-associated plasminogen activator (t-PA). uPA also plays a role in carcinogenesis: it is secreted in an inactive precursor form (pro-uPA) that binds with high affinity to a specific cell surface glycosylphosphatidylinositol-anchored receptor designated uPAR. The binding of pro-uPA to uPAR activates uPA and enhances the generation of plasmin at the cell surface, promoting matrix degradation, the activation of matrix metalloproteinases (MMPs) and growth factors, and the release of matrix- or cell-surface-anchored growth factors. PAI-1 is the physiological inhibitor of plasminogen activators and therefore controls the activation of plasminogen into plasmin (Fig. 7). PAI-1 interacts with the active form of uPAR-bound uPA, forming stable uPA-PAI complexes that are internalized [91].

In addition to interacting with uPA, PAI-1 binds to vitronectin leading to its stabilization. The binding of PAI-1 to vitronectin competes with the binding of integrins on the same site and thereby modulates cell migration. Integrins are heterodimeric cell surface proteins that on the one hand link the actin cytoskeleton to the cell membrane and, on the other, mediate cell-matrix interactions. Integrin signaling interacts with receptor tyrosine kinase signaling to regulate survival, proliferation and cell shape as well as polarity, adhesion, migration and differentiation. Although a high level of PAI-1 in tumor cells correlates with a more aggressive behavior in a number of tumor types [92], by decreasing the proteolytic activity in the extracellular matrix, PAI-1 prevents degradation of the matrix scaffold to which tumor cells need to adhere and through which they need to migrate [93]. This seemingly contradictory evidence points to the possiblity that PAI-1 may modulate tumor cell behavior through an alternative cellular mechanism [94].

The HLTF protein is involved in expression of the *PAI-1* gene by binding to a regulatory element (B-Box) in its promoter and activating its transcription in synergy with Sp1 or Sp3 [31]. Overexpression of HLTF causes a threefold induction of *PAI-1* transcription in HeLa cells, suggesting that the silencing of *HLTF* as the result of DNA methylation may attenuate PAI-1 expression. Nevertheless, several groups of researchers have demonstrated that PAI-1 expression increases with cervical, gastric and colorectal cancer stage and is associated with a poor prognosis [95–97]. As *HLTF* methylation increases early in the carcinogenic process, these data suggest that HLTF may not be involved in cancer-associated PAI-1 overexpression.

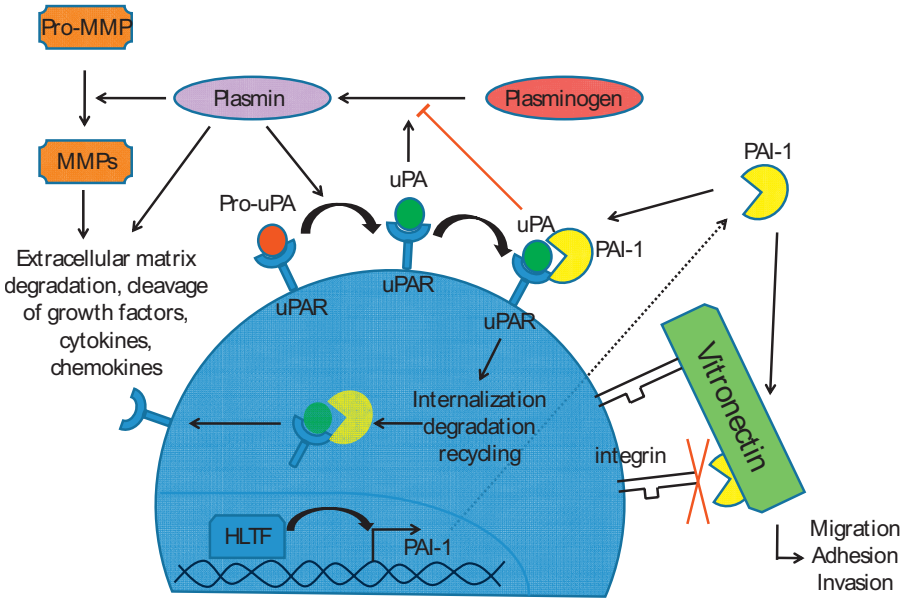

Figure 7. Schematic representation of the plasminogen-plasmin system (see text for explanations).

Conclusions

Very few data are available on the normal function of HLTF. Based on sequence similarities, HLTF was classified in the SWI/SNF family of chromatin/remodeling factors. In this family, HLTF is more closely related to the yeast RAD5 and RAD16 proteins [45] that are involved in DNA repair. However, an involvement of HLTF in this pathway has not yet been investigated. Several putative functions of the HLTF RING domain have been proposed: it could be involved in protein/protein interactions [98], in subnuclear trafficking [54] or ubiquitination [99]. The originality of HLTF compared to other SWI/SNF proteins is the presence of a DBD that targets it to specific gene sequences. Among these, a role of HLTF in transcription activation was demonstrated for the *PAI-1* gene, the β-globin LCR and the rabbit *uteroglobin* gene. HLTF was found to activate the PAI-1 promoter in synergy with Sp1 and Sp3 that could interact with its carboxyl-terminal domain. In addition, a chromatin immunoprecipitation experiment performed with an antibody raised against HLTF showed that it could bind to 3.3-kb repeated elements dispersed in heterochromatic regions of the human genome. Each of these repeats contains a double homeobox (*DUX*) gene [100]. One of these (*DUX4*) was found to express a transcription factor involved in a muscular dystrophy [101], but a role of HLTF in its expression was not investigated. Further studies of HLTF protein partners and targeted genes are needed to decipher its function in normal conditions and non-oncogenic pathologies.

Following recent publications on the subject, it now appears clear that HLTF is linked to the development and progression of cancer. HLTF is overexpressed very early in the carcinogenic process in an experimental model of estrogen-induced kidney tumors and is overexpressed in many immortalized cell lines. On the other hand, a number of studies have established that the frequency of *HLTF* promoter methylation increases drastically during the colorectal, gastric and cervical carcinogenic processes. Two hypotheses could be proposed to reconcile these apparently contradictory data. First, one could imagine that carcinogenesis requires inactivation of the *HLTF* gene, either by chromosomal deletion, promoter methylation or by a mutation affecting protein activity. Similar to what was described for TP53, some mutated HLTF proteins could escape degradation and accumulate in the cell, a phenomenon considered as overexpression [102]. In fact, some sequence discrepancies between HLTF cDNAs isolated from transformed cell lines and the reference genomic sequence in GenBank might provide support for this idea (e.g. P31R, found in www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=6596). In a second hypothesis, HLTF overexpression could initiate the transformation into cancer by changing the chromatin structure and activating the transcription of some target genes including *PAI-1*. Interestingly, another identified target gene encodes hTERT, the catalytic telomerase subunit whose expression allows infinite chromosome replications and hence proliferation of transformed cells [103]. However, HLTF expression would progressively become a burden for cell proliferation at later stages of cancer development, perhaps because of its role in differentiation shown during embryogenesis. At that stage, the tumor cells in which HLTF has been silenced by promoter

hypermethylation would release their full proliferation capacity. As a further complexity, one has to take into account the expression of HLTF protein variants, two of which are known [31] and others yet to be characterized, that might present different biological activities and varying expression levels during different steps of carcinogenesis. In fact, the short HLTFMet123 variant was the only one presenting transcriptional activity [31].

In conclusion, thorough studies on the function, expression levels and activity of HLTF proteins are needed, as well as the analysis of *HLTF* polymorphisms in control and cancer cells to decipher the role of this gene in cancer.

Acknowledgements. Research in the authors' laboratories was funded by the Fonds National pour la Recherche Scientifique (FNRS, Belgium; no. 7.4.641.06F to A.B.) and the Fonds pour la Recherche Médicale dans le Hainaut (A.B. and S.S.). G.D. and A.C. held pre-doctoral fellowships from Télévie (FNRS, Belgium) and the Fonds de Recherche pour l'Industrie et l'Agriculture (FRIA, Belgium), respectively. We thank A. Lechien for his help in the realization of the illustrations and R. Shelby for correcting the English.

1 Kornberg, R. D. and Lorch, Y. (1999) Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. Cell 98, 285–294.
2 Owen-Hughes, T. (2003) Colworth Memorial Lecture: Pathways for remodelling chromatin. Biochem. Soc. Trans. 31, 893–905.
3 Mohrmann, L. and Verrijzer, C. P. (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Biochim. Biophys. Acta 1681, 59–73.
4 Li, B., Carey, M. and Workman, J. L. (2007) The role of chromatin during transcription. Cell, 128, 707–719.
5 5Lorch, Y., Zhang, M. and Kornberg, R. D. (1999) Histone octamer transfer by a chromatin-remodeling complex. Cell 96, 389–392.
6 Roberts, C. W. and Orkin, S. H. (2004) The SWI/SNF complex – chromatin and cancer. Nat. Rev. Cancer 4, 133–142.
7 Biegel, J. A., Zhou, J. Y., Rorke, L. B., Stenstrom, C., Wainwright, L. M. and Fogelgren, B. (1999) Germ-line and acquired mutations of INI1 in atypical teratoid and rhabdoid tumors. Cancer Res. 59, 74–79.
8 Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M. F., Ambros, P., Handgretinger, R., Aurias, A. and Delattre, O. (1998) Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. Nature 394, 203–206.
9 Roberts, C. W., Leroux, M. M., Fleming, M. D. and Orkin, S. H. (2002) Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene Snf5. Cancer Cell 2, 415–425.
10 Bai, A. H., Tong, J. H., To, K. F., Chan, M. W., Man, E. P., Lo, K. W., Lee, J. F., Sung, J. J. and Leung, W. K. (2004) Promoter hypermethylation of tumor-related genes in the progression of colorectal neoplasia. Int. J. Cancer 112, 846–853.
11 Brandes, J. C., van Engeland, M., Wouters, K. A., Weijenberg, M. P. and Herman, J. G. (2005) CHFR promoter hypermethylation in colon cancer correlates with the microsatellite instability phenotype. Carcinogenesis 26, 1152–1156.
12 Hibi, K., Nakayama, H., Kanyama, Y., Kodera, Y., Ito, K., Akiyama, S. and Nakao, A. (2003) Methylation pattern of HLTF. gene in digestive tract cancers. Int. J. Cancer 104, 433–436.
13 Hibi, K., Kodera, Y., Ito, K., Akiyama, S. and Nakao, A. (2005) Aberrant methylation of HLTF, SOCS-1 and CDH13 genes is shown in colorectal cancers without lymph node metastasis. Dis. Colon Rectum 48, 1282–1286.
14 Hibi, K. and Nakao, A. (2006) Highly-methylated colorectal cancers show poorly-differentiated phenotype. Anticancer Res. 26, 4263–4266.
15 Hibi, K. and Nakao, A. (2006) Lymph node metastasis is infrequent in patients with highly-methylated colorectal cancer. Anticancer Res. 26, 55–58.
16 Kim, Y. H., Petko, Z., Dzieciatkowski, S., Lin, L., Ghiassi, M., Stain, S., Chapman, W. C., Washington, M. K., Willis, J., Markowitz, S. D. and Grady, W. M. (2006) CpG island methylation of genes accumulates during the adenoma progression step of the multistep pathogenesis of colorectal cancer. Genes Chromosom. Cancer 45, 781–789.
17 Leung, W. K., To, K. F., Man, E. P., Chan, M. W., Bai, A. H., Hui, A. J., Chan, F. K., Lee, J. F. and Sung, J. J. (2004) Detection of epigenetic changes in fecal DNA as a molecular screening test for colorectal cancer: a feasibility study. Clin. Chem. 50, 2179–2182.
18 Leung, W. K., To, K. F., Man, E. P., Chan, M. W., Bai, A. H., Hui, A. J., Chan, F. K. and Sung, J. J. (2005) Quantitative detection of promoter hypermethylation in multiple genes in the serum of patients with colorectal cancer. Am. J. Gastroenterol. 100, 2274–2279.
19 Leung, W. K., To, K. F., Man, E. P., Chan, M. W., Hui, A. J., Ng, S. S., Lau, J. Y. and Sung, J. J. (2007) Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in fecal samples of patients with colorectal cancer or polyps. Am. J. Gastroenterol. 102, 1070–1076.
20 Moinova, H. R., Chen, W. D., Shen, L., Smiraglia, D., Olechnowicz, J., Ravi, L., Kasturi, L., Myeroff, L., Plass, C., Parsons, R., Minna, J., Willson, J. K., Green, S. B., Issa, J. P. and Markowitz, S. D. (2002) HLTF gene silencing in human colon cancer. Proc. Natl. Acad. Sci. USA, 99, 4562–4567.
21 Kang, M. Y., Lee, B. B., Kim, Y. H., Chang, D. K., Kyu, P. S., Chun, H. K., Song, S. Y., Park, J. and Kim, D. H. (2007) Association of the SUV39H1 histone methyltransferase with the DNA methyltransferase 1 at mRNA expression level in primary colorectal cancer. Int. J. Cancer 121, 2192–2197.
22 Hamai, Y., Oue, N., Mitani, Y., Nakayama, H., Ito, R., Matsusaki, K., Yoshida, K., Toge, T. and Yasui, W. (2003) DNA hypermethylation and histone hypoacetylation of the HLTF gene are associated with reduced expression in gastric carcinoma. Cancer Sci. 94, 692–698.
23 Kim, J. J., Chung, S. W., Kim, J. H., Kim, J. W., Oh, J. S., Kim, S., Song, S. Y., Park, J. and Kim, D. H. (2006) Promoter methylation of helicase-like transcription factor is associated with the early stages of gastric cancer with family history. Ann. Oncol. 17, 657–662.
24 Leung, W. K., Yu, J., Bai, A. H., Chan, M. W., Chan, K. K., To, K. F., Chan, F. K., Ng, E. K., Chung, S. C. and Sung, J. J. (2003) Inactivation of helicase-like transcription factor by promoter hypermethylation in human gastric cancer. Mol. Carcinog. 37, 91–97.
25 Oue, N., Mitani, Y., Motoshita, J., Matsumura, S., Yoshida, K., Kuniyasu, H., Nakayama, H. and Yasui, W. (2006) Accumulation of DNA methylation is associated with tumor stage in gastric cancer. Cancer 106, 1250–1259.
26 Fukuoka, T., Hibi, K. and Nakao, A. (2006) Aberrant methylation is frequently observed in advanced esophageal squamous cell carcinoma. Anticancer Res. 26, 3333–3335.
27 Kang, S., Kim, J. W., Kang, G. H., Lee, S., Park, N. H., Song, Y. S., Park, S. Y., Kang, S. B. and Lee, H. P. (2006) Comparison of DNA hypermethylation patterns in different types of uterine cancer: cervical squamous cell carcinoma, cervical adenocarcinoma and endometrial adenocarcinoma. Int. J. Cancer, 118, 2168–2171.
28 Jo, H., Kang, S., Kim, J. W., Kang, G. H., Park, N. H., Song, Y. S., Park, S. Y., Kang, S. B. and Lee, H. P. (2007) Hypermethylation of the COX-2 gene is a potential prognostic

marker for cervical cancer. J. Obstet. Gynaecol. Res. 33, 236–241.
29 Debauve, G., Nonclercq, D., Ribaucour, F., Wiedig, M., Gerbaux, C., Leo, O., Laurent, G., Journe, F., Belayew, A. and Toubeau, G. (2006) Early expression of the helicase-like transcription factor (HLTF/SMARCA3) in an experimental model of estrogen-induced renal carcinogenesis. Mol. Cancer 5, 23.
30 Sheridan, P. L., Schorpp, M., Voz, M. L. and Jones, K. A. (1995) Cloning of an SNF2/SWI2-related protein that binds specifically to the SPH motifs of the SV40 enhancer and to the HIV-1 promoter. J. Biol. Chem. 270, 4575–4587.
31 Ding, H., Descheemaeker, K., Marynen, P., Nelles, L., Carvalho, T., Carmo-Fonseca, M., Collen, D. and Belayew, A. (1996) Characterization of a helicase-like transcription factor involved in the expression of the human plasminogen activator inhibitor-1 gene. DNA Cell Biol. 15, 429–442.
32 Zhang, Q., Ekhterae, D. and Kim, K. H. (1997) Molecular cloning and characterization of P113, a mouse SNF2/SWI2-related transcription factor. Gene 202, 31–37.
33 Hayward-Lester, A., Hewetson, A., Beale, E. G., Oefner, P. J., Doris, P. A. and Chilton, B. S. (1996) Cloning, characterization, and steroid-dependent posttranscriptional processing of RUSH-1 alpha and beta, two uteroglobin promoter-binding proteins. Mol. Endocrinol. 10, 1335–1349.
34 Gong, X., Kaushal, S., Ceccarelli, E., Bogdanova, N., Neville, C., Nguyen, T., Clark, H., Khatib, Z. A., Valentine, M., Look, A. T. and Rosenthal, N. (1997) Developmental regulation of Zbu1, a DNA-binding member of the SWI2/SNF2 family. Dev. Biol. 183, 166–182.
35 Mahajan, M. C. and Weissman, S. M. (2002) DNA-dependent adenosine triphosphatase (helicaselike transcription factor) activates beta-globin transcription in K562 cells. Blood 99, 348–356.
36 Ding, H., Benotmane, A. M., Suske, G., Collen, D. and Belayew, A. (1999) Functional interactions between Sp1 or Sp3 and the helicase-like transcription factor mediate basal expression from the human plasminogen activator inhibitor-1 gene. J. Biol. Chem. 274, 19573–19580.
37 Hewetson, A. and Chilton, B. S. (2003) An Sp1-NF-Y/progesterone receptor DNA binding-dependent mechanism regulates progesterone-induced transcriptional activation of the rabbit RUSH/SMARCA3 gene. J. Biol. Chem. 278, 40177–40185.
38 Hewetson, A., Hendrix, E. C., Mansharamani, M., Lee, V. H. and Chilton, B. S. (2002) Identification of the RUSH consensus-binding site by cyclic amplification and selection of targets: demonstration that RUSH mediates the ability of prolactin to augment progesterone-dependent gene expression. Mol. Endocrinol. 16, 2101–2112.
39 Bole-Feysot, C., Goffin, V., Edery, M., Binart, N. and Kelly, P. A. (1998) Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. Endocr. Rev. 19, 225–268.
40 Hewetson, A., Moore, S. L. and Chilton, B. S. (2004) Prolactin signals through RUSH/SMARCA3 in the absence of a physical association with Stat5a. Biol. Reprod. 71, 1907–1912.
41 Lin, Y., Sheridan, P. L., Jones, K. A. and Evans, G. A. (1995) The HIP116 SNF2/SW12-related transcription factor gene (SNF2L3) is located on human chromosome 3q25.1-q26.1. Genomics 27, 381–382.
42 Iyer, L. M., Babu, M. M. and Aravind, L. (2006) The HIRAN domain and recruitment of chromatin remodeling and repair activities to damaged DNA. Cell Cycle 5, 775–782.
43 Eisen, J. A., Sweder, K. S. and Hanawalt, P. C. (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. Nucleic Acids Res., 23, 2715–2723.
44 Linder, B., Cabot, R. A., Schwickert, T. and Rupp, R. A. (2004) The SNF2 domain protein family in higher vertebrates displays dynamic expression patterns in *Xenopus laevis* embryos. Gene 326, 59–66.
45 Flaus, A., Martin, D. M., Barton, G. J. and Owen-Hughes, T. (2006) Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. Nucleic Acids Res. 34, 2887–2905.
46 Chen, S., Davies, A. A., Sagan, D. and Ulrich, H. D. (2005) The RING finger ATPase Rad5p of *Saccharomyces cerevisiae* contributes to DNA double-strand break repair in a ubiquitin-independent manner. Nucleic Acids Res. 33, 5878–5886.
47 Hoege, C., Pfander, B., Moldovan, G. L., Pyrowolakis, G. and Jentsch, S. (2002) RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419, 135–141.
48 Ramsey, K. L., Smith, J. J., Dasgupta, A., Maqani, N., Grant, P. and Auble, D. T. (2004) The NEF4 complex regulates Rad4 levels and utilizes Snf2/Swi2-related ATPase activity for nucleotide excision repair. Mol. Cell Biol. 24, 6362–6378.
49 Yu, S., Owen-Hughes, T., Friedberg, E. C., Waters, R. and Reed, S. H. (2004) The yeast Rad7/Rad16/Abf1 complex generates superhelical torsion in DNA that is required for nucleotide excision repair. DNA Repair (Amst.) 3, 277–287.
50 Morris, J. R., Pangon, L., Boutell, C., Katagiri, T., Keep, N. H. and Solomon, E. (2006) Genetic analysis of BRCA1 ubiquitin ligase activity and its relationship to breast cancer susceptibility. Hum. Mol. Genet. 15, 599–606.
51 Ulrich HD (2003) Protein-protein interactions within an E2-RING finger complex: implications for ubiquitin-dependent DNA damage repair. J. Biol. Chem. 278, 7051–7058.
52 Xia, Y., Pao, G. M., Chen, H. W., Verma, I. M. and Hunter, T. (2003) Enhancement of BRCA1 E3 ubiquitin ligase activity through direct interaction with the BARD1 protein. J. Biol. Chem. 278, 5255–5263.
53 Devine, J. H., Hewetson, A., Lee, V. H. and Chilton, B. S. (1999) After chromatin is SWItched-on can it be RUSHed? Mol. Cell Endocrinol. 151, 49–56.
54 Mansharamani, M., Hewetson, A. and Chilton, B. S. (2001) Cloning and characterization of an atypical type IV P-type ATPase that binds to the RING motif of RUSH transcription factors. J. Biol. Chem. 276, 3641–3649.
55 Hanahan, D. and Weinberg, R. A. (2000) The hallmarks of cancer. Cell 100, 57–70.
56 Chuang, J. C. and Jones, P. A. (2007) Epigenetics and MicroRNAs. Pediatr. Res. 61. 24R–29R
57 Klose, R. J. and Bird, A. P. (2006) Genomic DNA methylation: the mark and its mediators. Trends Biochem. Sci. 31, 89–97.
58 Esteller, M. (2005) Aberrant DNA methylation as a cancer-inducing mechanism. Annu. Rev. Pharmacol. Toxicol. 45, 629–656.
59 Esteller, M. (2007) Cancer epigenomics: DNA methylomes and histone-modification maps. Nat. Rev. Genet. 8, 286–298.
60 Bird, A. (2002) DNA methylation patterns and epigenetic memory. Genes Dev., 16, 6–21.
61 Maley, C. C., Galipeau, P. C., Li, X., Sanchez, C. A., Paulson, T. G. and Reid, B. J. (2004) Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. Cancer Res. 64, 3414–3427.
62 Toyota, M. and Issa, J. P. (2000) The role of DNA hypermethylation in human neoplasia. Electrophoresis 21, 329–333.
63 Issa, J. P., Shen, L. and Toyota, M. (2005) CIMP, at last. Gastroenterology 129, 1121–1124.
64 Samowitz, W. S., Sweeney, C., Herrick, J., Albertsen, H., Levin, T. R., Murtaugh, M. A., Wolff, R. K. and Slattery, M. L. (2005) Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. Cancer Res. 65, 6063–6069.
65 Bariol, C., Suter, C., Cheong, K., Ku, S. L., Meagher, A., Hawkins, N. and Ward, R. (2003) The relationship between hypomethylation and CpG island methylation in colorectal neoplasia. Am. J. Pathol. 162, 1361–1371.

66 Rashid, A., Shen, L., Morris, J. S., Issa, J. P. and Hamilton, S. R. (2001) CpG island methylation in colorectal adenomas. Am. J. Pathol. 159, 1129–1135.
67 Jen, J., Wu, L. and Sidransky, D. (2000) An overview on the isolation and analysis of circulating tumor DNA in plasma and serum. Ann. N. Y. Acad. Sci. 906, 8–12.
68 Ahlquist, D. A., Skoletsky, J. E., Boynton, K. A., Harrington, J. J., Mahoney, D. W., Pierceall, W. E., Thibodeau, S. N. and Shuber, A. P. (2000) Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. Gastroenterology 119, 1219–1227.
69 Herman, J. G., Graff, J. R., Myohanen, S., Nelkin, B. D. and Baylin, S. B. (1996) Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc. Natl. Acad. Sci. USA 93, 9821–9826.
70 Widschwendter, M. and Menon, U. (2006) Circulating methylated DNA: a new generation of tumor markers. Clin. Cancer Res. 12, 7205–7208.
71 Wallner, M., Herbst, A., Behrens, A., Crispin, A., Stieber, P., Goke, B., Lamerz, R. and Kolligs, F. T. (2006) Methylation of serum DNA is an independent prognostic marker in colorectal cancer. Clin. Cancer Res. 12, 7347–7352.
72 Lenhard, K., Bommer, G. T., Asutay, S., Schauer, R., Brabletz, T., Goke, B., Lamerz, R. and Kolligs, F. T. (2005) Analysis of promoter methylation in stool: a novel method for the detection of colorectal cancer. Clin. Gastroenterol. Hepatol. 3, 142–149.
73 Leung, W. K., To, K. F., Man, E. P., Chan, M. W., Bai, A. H., Hui, A. J., Chan, F. K., Lee, J. F. and Sung, J. J. (2004) Detection of epigenetic changes in fecal DNA as a molecular screening test for colorectal cancer: a feasibility study. Clin. Chem. 50, 2179–2182.
74 Muller, H. M., Oberwalder, M., Fiegl, H., Morandell, M., Goebel, G., Zitt, M., Muhlthaler, M., Ofner, D., Margreiter, R. and Widschwendter, M. (2004) Methylation changes in faecal DNA: a marker for colorectal cancer screening? Lancet 363, 1283–1285.
75 Petko, Z., Ghiassi, M., Shuber, A., Gorham, J., Smalley, W., Washington, M. K., Schultenover, S., Gautam, S., Markowitz, S. D. and Grady, W. M. (2005) Aberrantly methylated CDKN2A, MGMT, and MLH1 in colon polyps and in fecal DNA from patients with colorectal polyps. Clin. Cancer Res. 11, 1203–1209.
76 Fuchs, C. S. and Mayer, R. J. (1995) Gastric carcinoma. N. Engl. J. Med. 333, 32–41.
77 Kang, G. H., Lee, S., Kim, J. S. and Jung, H. Y. (2003) Profile of aberrant CpG island methylation along the multistep pathway of gastric carcinogenesis. Lab. Invest. 83, 635–641.
78 Oue, N., Shigeishi, H., Kuniyasu, H., Yokozaki, H., Kuraoka, K., Ito, R. and Yasui, W. (2001) Promoter hypermethylation of MGMT is associated with protein loss in gastric carcinoma. Int. J. Cancer 93, 805–809.
79 Kondo, T., Oue, N., Yoshida, K., Mitani, Y., Naka, K., Nakayama, H. and Yasui, W. (2004) Expression of POT1 is associated with tumor stage and telomere length in gastric carcinoma. Cancer Res. 64, 523–529.
80 Brock, M. V., Gou, M., Akiyama, Y., Muller, A., Wu, T. T., Montgomery, E., Deasel, M., Germonpre, P., Rubinson, L., Heitmiller, R. F., Yang, S. C., Forastiere, A. A., Baylin, S. B. and Herman, J. G. (2003) Prognostic importance of promoter hypermethylation of multiple genes in esophageal adenocarcinoma. Clin. Cancer Res. 9, 2912–2919.
81 Oshimo, Y., Oue, N., Mitani, Y., Nakayama, H., Kitadai, Y., Yoshida, K., Chayama, K. and Yasui, W. (2004) Frequent epigenetic inactivation of RIZ1 by promoter hypermethylation in human gastric carcinoma. Int. J. Cancer 110, 212–218.
82 Oue, N., Oshimo, Y., Nakayama, H., Ito, R., Yoshida, K., Matsusaki, K. and Yasui, W. (2003) DNA methylation of multiple genes in gastric carcinoma: association with histological type and CpG island methylator phenotype. Cancer Sci. 94, 901–905.
83 Toyota, M., Ahuja, N., Suzuki, H., Itoh, F., Ohe-Toyota, M., Imai, K., Baylin, S. B. and Issa, J. P. (1999) Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. Cancer Res. 59, 5438–5442.
84 Hollstein, M. C., Metcalf, R. A., Welsh, J. A., Montesano, R. and Harris, C. C. (1990) Frequent mutation of the p53 gene in human esophageal cancer. Proc. Natl. Acad. Sci. USA 87, 9958–9961.
85 Jiang, W., Zhang, Y. J., Kahn, S. M., Hollstein, M. C., Santella, R. M., Lu, S. H., Harris, C. C., Montesano, R. and Weinstein, I. B. (1993) Altered expression of the cyclin D1 and retinoblastoma genes in human esophageal cancer. Proc. Natl. Acad. Sci. USA 90, 9026–9030.
86 Tsuda, T., Nakatani, H., Tahara, E., Sakamoto, H., Terada, M. and Sugimura, T. (1989) HST1 and INT2 gene coamplification in a squamous cell carcinoma of the gallbladder. Jpn. J. Clin. Oncol. 19, 26–29.
87 Zhou, X., Tarmin, L., Yin, J., Jiang, H. Y., Suzuki, H., Rhyu, M. G., Abraham, J. M. and Meltzer, S. J. (1994) The MTS1 gene is frequently mutated in primary human esophageal tumors. Oncogene 9, 3737–3741.
88 Hibi, K., Taguchi, M., Nakayama, H., Takase, T., Kasai, Y., Ito, K., Akiyama, S. and Nakao, A. (2001) Molecular detection of p16 promoter methylation in the serum of patients with esophageal squamous cell carcinoma. Clin. Cancer Res. 7, 3135–3138.
89 Cullen, A. P., Reid, R., Campion, M. and Lorincz, A. T. (1991) Analysis of the physical state of different human papillomavirus DNAs in intraepithelial and invasive cervical neoplasm. J. Virol. 65, 606–612.
90 Dong, S. M., Kim, H. S., Rha, S. H. and Sidransky, D. (2001) Promoter hypermethylation of multiple genes in carcinoma of the uterine cervix. Clin. Cancer Res. 7, 1982–1986.
91 Olson, D., Pollanen, J., Hoyer-Hansen, G., Ronne, E., Sakaguchi, K., Wun, T. C., Appella, E., Dano, K. and Blasi, F. (1992) Internalization of the urokinase-plasminogen activator inhibitor type-1 complex is mediated by the urokinase receptor. J. Biol. Chem. 267, 9129–9133.
92 Steinmetzer, T. (2003) Synthetic urokinase inhibitors as potential antitumor drugs. IDrugs. 6, 138–146.
93 Schmitt, M., Harbeck, N., Thomssen, C., Wilhelm, O., Magdolen, V., Reuning, U., Ulm, K., Hofler, H., Janicke, F. and Graeff, H. (1997) Clinical impact of the plasminogen activation system in tumor invasion and metastasis: prognostic relevance and target for therapy. Thromb. Haemost. 78, 285–296.
94 Fen, L. C., Kandel, C., Baliko, F., Nadesan, P., Brunner, N. and Alman, B. A. (2005) Plasminogen activator inhibitor-1 (PAI-1) modifies the formation of aggressive fibromatosis (desmoid tumor). Oncogene 24, 1615–1624.
95 Sakakibara, T., Hibi, K., Koike, M., Fujiwara, M., Kodera, Y., Ito, K. and Nakao, A. (2005) Plasminogen activator inhibitor-1 as a potential marker for the malignancy of colorectal cancer. Br. J. Cancer 93, 799–803.
96 Sakakibara, T., Hibi, K., Koike, M., Fujiwara, M., Kodera, Y., Ito, K. and Nakao, A. (2006) Plasminogen activator inhibitor-1 as a potential marker for the malignancy of gastric cancer. Cancer Sci. 97, 395–399.
97 Hazelbag, S., Kenter, G. G., Gorter, A. and Fleuren, G. J. (2004) Prognostic relevance of TGF-beta1 and PAI-1 in cervical cancer. Int. J. Cancer 112, 1020–1028.
98 Saurin, A. J., Borden, K. L., Boddy, M. N. and Freemont, P. S. (1996) Does this have a familiar RING? Trends Biochem. Sci. 21, 208–214.
99 Li, W., Tu, D., Brunger, A. T. and Ye, Y. (2007) A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate. Nature 446, 333–337.
100 Ding, H., Beckers, M. C., Plaisance, S., Marynen, P., Collen, D. and Belayew, A. (1998) Characterization of a double homeodomain protein (DUX1) encoded by a cDNA homologous to 3.3 kb dispersed repeated elements. Hum. Mol. Genet. 7, 1681–1694.

101 Dixit, M., Ansseau, E., Tassin, A., Winokur, S., Shi, R., Qian, H., Sauvage, S., Mattéotti, C., Van Acker, A., Leo, O., Figlewicz, D., Barro, M., Laoudj-Chenivesse, D., Belayew, A., Coppée, F. and Chen, Y. W. (2007) *DUX4*, a candidate gene of facioscapulohumeral muscular dystrophy, encodes a transcriptional activator of *PITX1*. Proc. Natl. Acad. Sci. U.S.A, 104, 18157–18162.

102 Szymanska, K. and Hainaut, P. (2003) TP53 and mutations in human cancer. Acta Biochim. Pol. 50, 231–238.

103 Androphy, E., Doshi, N. and Belayew, A. (2003) Diagnosis and treatment of cancer. New England Medical Center and University of Mons-Hainaut. [7,183,051]. Boston, MA; Mons, BE. 27-6-2002.