The role of epigenetic inactivation of *14-3-3σ* in human cancer

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ABSTRACT

Cancer cells show characteristic alterations in DNA methylation patterns. Aberrant CpG methylation of specific promoters results in inactivation of tumor suppressor genes and therefore plays an important role in carcinogenesis. The p53-regulated gene *14-3-3σ* undergoes frequent epigenetic silencing in several types of cancer, including carcinoma of the breast, prostate, and skin, suggesting that the loss of 14-3-3σ expression may be causally involved in tumor progression. Functional studies demonstrated that 14-3-3σ is involved in cell-cycle control and prevents the accumulation of chromosomal damage. The recent identification of novel 14-3-3σ-associated proteins by a targeted proteomics approach implies that 14-3-3σ regulates diverse cellular processes, which may become deregulated after silencing of *14-3-3σ* expression in cancer cells.

Keywords: 14-3-3σ, CpG methylation, p53, epigenetic silencing, cancer, cell cycle.

INTRODUCTION

In the process of neoplastic transformation changes in the function of critical genes which allow cells to overgrow their neighboring cells are selected for. The altered genes which are causally involved in carcinogenesis can be broadly divided in two major classes: oncogenes and tumor suppressor genes. Whereas oncogenes gain an increased function via activating point mutations or gene amplification, tumor suppressor genes lose their function in cancer cells. Tumor suppressor genes may be subdivided into caretakers and gatekeepers [1]. Caretaker genes encode proteins which function in the preservation of genomic integrity, whereas gatekeepers keep cells in a defined state of differentiation.

The inactivation of tumor suppressor genes is mediated by two mechanisms: genetic inactivation by DNA mutations and epigenetic silencing. In some cases genetic and epigenetic events complement each other (Fig. 1). In general bi-allelic gene inactivation resulting in complete loss of function is characteristic for tumor suppressor genes. This review will focus on the epigenetic inactivation of *14-3-3σ* gene expression in human cancers and possible functional implications of the loss of 14-3-3σ function for the progression of tumors.

EPIGENETIC SILENCING IN CANCER

The term *epigenetic* refers to information which is transmitted from the parental genome to the next generation of cells which is not encoded by the primary DNA sequence. Epigenetic mechanisms are essential for the regulation of gene expression and genome integrity in normal cells (for reviews see [2-4]). Epigenetic information is often transmitted by methylation of the 5 carbon position of cytosine within a CpG dinucleotide, also referred to as DNA methylation. CpG dinucleotides are under-represented in the genome, but over-represented in short regions of 500–4,000 bp (base pair) in length, known as CpG islands, which are rich in CpG content [3, 5]. CpG islands are present in the proximal promoter regions of about 60% of the genes in the mammalian genome and are, generally, unmethylated in normal cells.

Patterns of DNA methylation and chromatin structure are profoundly altered in neoplasia and include genome-wide losses of and regional gains in DNA methylation [6]. Global hypomethylation was shown to cause genomic instability which in turn may promote secondary genetic alterations [7, 8], whereas local hypermethylation of promoter regions is associated with transcriptional silencing

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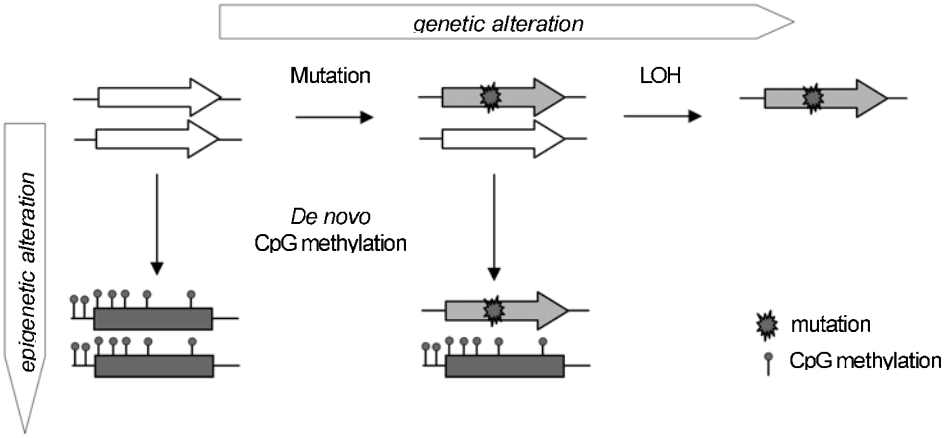

Fig. 1 Mechanisms of tumor suppressor gene inactivation in cancer. In diploid cells one allele of a tumor suppressor gene can be inactivated by a point mutation. The remaining allele is frequently lost due to deletion of the gene, also designated as loss of heterozygosity (LOH). Alternatively, the second allele can undergo *de novo* methylation and therefore become transcriptionally silent. It is also possible, that both alleles are silenced by aberrant promoter methylation.

and can lead to the loss of tumor-suppressor gene function. The molecular mechanisms of epigenetic silencing during tumor formation are only partially understood. In normal cells the pattern of CpG methylation is established by two types of methyltransferases catalyzing the addition of a methyl-group at the C-5 position of cytosine: DNMT1 serves as a maintenance methyltransferase, whereas DNMT3A and DNMT3B mediate *de novo* DNA methylation. All three DNMTs are modestly over-expressed at the mRNA and/or protein level in many types of tumor cells [9, 10]. Ectopic expression of DNMT1 cooperates with oncogenes in the transformation of primary cells supporting the idea that aberrant expression of DNA methyltransferases may contribute to abnormal promoter methylation in cancer cells [11] .

Factors directing the activity of DNMTs to specific genomic sites under normal and pathological conditions remained largely unknown. Covalent modifications of core histones may serve as plausible marks for the establishment of DNA methylation. The methylation of histone H3 lysine 9 was shown to be a prerequisite for DNA methylation in *Neurospora crassa* and *Arabidopsis thaliana* [12, 13]. Although this requirement does not seem to be conserved in mammalian cells, the concept that histone methylation provides a signal which somehow can be interpreted by DNA methylation machinery is supported by experimental data. For example, the histone methyltransferase Suv39H1 directs DNA methylation to satellite repeats at pericentric heterochromatin [14]. The $p16^{INK4A}$ gene has been shown to undergo re-activation after reversal of DNA methylation after experimental removal of *DNMT* activity in colorectal cancer cells [15]. Subsequent re-silencing of $p16^{INK4A}$ occurred after prolonged passage of these cells. Methylation of histone H3-K9 was detected in association with re-silencing of this tumor suppressor gene in the absence of DNA methylation [15]. Therefore, the methylation of histones may precede methylation of DNA during epigenetic silencing of tumor suppressor genes. In plants and some animals double stranded RNAs were implicated in the guidance of *de novo* methylation [16, 17]. Also, RNAi-mediated transcriptional silencing has been associated with promoter methylation in human cells [18, 19]. In addition, transcription factors which interact with DNA in a sequence-specific manner may direct CpG methylation to specific promoters. In line with this hypothesis, it was recently shown that the Myc protein recruits DNMT3A methyltransferase to the $p21^{WAF1}$ promoter and represses its expression through CpG methylation [20]. However, so far it is unclear whether any of these mechanisms plays a role in establishing the aberrant methylation patterns seen in human malignancies.

Methylated CpG-dinucleotides are bound by the methyl-CpG-binding domain (MBD) containing proteins MBD1, MBD2, MBD3, MBD4 and MeCP2. The protein Kaiso binds to methylated CpG groups via its zinc-finger motif. With the exception of MBD4, which operates in mismatch repair, these proteins were shown to recruit chromatin remodeling factors (e.g. the Mi2/NuRD complex) and histone deacetylases (HDAC) to the respective promoters and thereby establish transcriptionally inactive chromatin (Fig. 2). How and at which stage of tumor progression CpG methylation of tumor suppressive genes is established

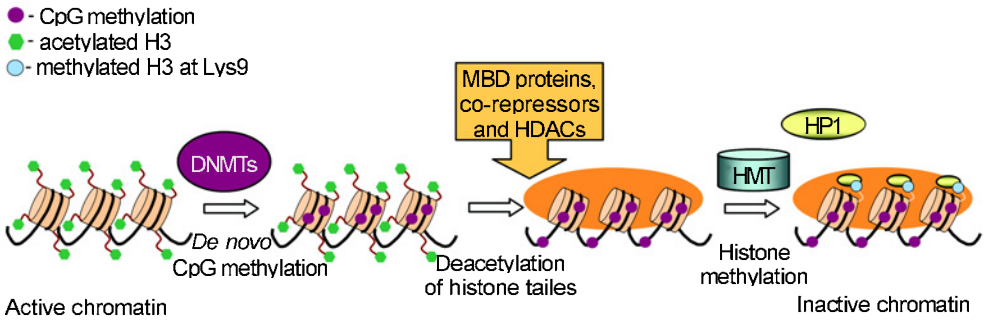

Fig. 2 The relationship between DNA methylation and chromatin structure. The carbon atom at the 5' position of cytosines within a CpG-dinucleotide context can be methylated by DNA methyltransferases (DNMTs). Clusters of methylated CpGs are recognized and bound by specific proteins sharing a methyl-CpG binding domain (MBD). In turn, MBD proteins recruit transcriptional co-repressors, chromatin remodeling complexes and histone deacetylases (HDACs). Deacetylation of histone tails (N-terminal parts of histone H3 and H4) results in alteration of nucleosomal structure and decrease in transcriptional activity of the chromatin. Subsequent methylation of histone tails by histone methyltransferases (HMTs) (particularly, lysine 9 of H3 and lysine 20 of H4) and recruitment of auxiliary proteins, such as heterochromatin binding protein 1 (HP1), enhance the formation of transcriptionally incompetent heterochromatin.

is unknown. Stochastic, age-associated accumulation of aberrantly methylated CpG-sites may be involved in this process.

Epigenetic inactivation of tumor suppressor genes significantly contributes to tumor development [21]. The genes aberrantly methylated in human cancers fall into functional categories intimately involved in cellular processes relevant to carcinogenesis such as DNA repair (*MGMT, MLH1, BRCA1*), cell cycle control (CDK inhibitors, *p15, p16, p27*), apoptosis (*APAF1, CASP8*), invasion (*CDH1*, *TIMP3*) and angiogenesis (*THBS1, VHL*). Nearly 50% of the genes that cause familial forms of cancer when mutated in the germ line are known to undergo methylation-associated silencing in various sporadic forms of cancer [21]. Although some methylation changes seem to be common to many kinds of cancer, tissue-specific differences exist. For example, the $p15^{INK4B}$ promoter is frequently methylated in lymphoid, but not in solid cancers [22], and *glutathione S-transferase p 1* (*GSTP1*) is methylated in prostate, breast and hepatic cancers, but rarely in others [23].

Experimental reversion of DNA methylation leads to re-expression of silenced genes in tumor cells, which may have consequences at the cellular level: e.g. restored sensitivity to apoptotic stimuli after reactivation of *caspase 8* [24] or inhibition of cell-proliferation after re-expression of the CDK-inhibitor *p16* [25]. Consistent with a role of hypermethylation-mediated gene repression in tumor formation, inactivation of *DNMT1* or *MBD2* suppresses intestinal tumorigenesis in Apc^{min} mice [26, 27].

Although CpG methylation patterns can be erased during DNA replication in the absence of DNMT activity, aberrant epigenetic marks persist through multiple generations of cells and are as stable as genetic alterations. For example most cancer cell lines maintain the pattern of aberrant DNA methylation characteristic for primary tumors of the same tissue origin [28]. Moreover, aberrant methylation of CpG-islands, also called epimutation, can be transmitted through the germ line, as recently shown for the *MLH1* gene [29].

SILENCING OF *14-3-3σ* IN CANCER

The p53-regulated *14-3-3σ* gene displays a number of properties (reviewed in [30] and discussed in the next section), which suggest that this gene may function in tumor suppression. SAGE analysis revealed down-regulation of *14-3-3σ* in breast cancer cells [31]. A subsequent search for the mutations in the *14-3-3σ* locus did not reveal any genetic alterations which could explain its down-regulation. Instead the epigenetic silencing by CpG methylation turned out to be responsible for the loss or reduction of *14-3-4σ* expression in more than 90% of ~90 analyzed primary breast cancer samples [32]. The treatment of breast cancer cells with an inhibitor of DNA methyltransferases, 5-aza-2'-deoxycytidine (5Aza-2'dC), results in re-activation of *14-3-3σ* expression, indicating a causal role for hypermethylation in the loss of *14-3-3σ* expression [32]. This study provided the first example for loss of *14-3-3σ* expression by epigenetic silencing in primary tumors. Subsequently, numerous reports documented silencing of *14-3-3σ* gene associated with CpG methylation in several common human malignancies, with a particularly high prevalence in prostate, breast and clear cell type ovarian carcinomas (summarized in the Tab. 1). In

Tab. 1 Silencing of *14-3-3σ* in different types of epithelial tumors

Organ	Frequency of CpG methylation	Expression	Ref.
Mammary gland	86% (43/50) primary tumors 100% (32/32) microdissected carcinoma	mRNA down-regulation in 96% (43/45) primary tumors	[32]
	96% (24/25) invasive ductal carcinoma 83% (15/18) ductal carcinoma *in situ* 38% (3/8) atypical hyperplasia	ND	[37]
	ND	Protein down-regulation in 77% (55/71) invasive ductal carcinoma 35% (12/34) ductal carcinoma *in situ* 8% (24/26) usual ductal hyperplasia	[51]
	ND	Protein down-regulation in 77% (33/43) primary breast carcinoma	[45]
Stomach	43% (26/60) primary gastric cancer	mRNA down-regulation in cell lines positive for the methylation	[52]
Liver	89% (17/19) primary HCC	Protein down-regulation in 88% (14/16) primary HCC	[39]
	60% (47/79) intrahepatic cholangiocarcinoma	ND	[53]
Lung	33% (/24) microdissected primary SCLC 69% (9/13) SCLC cell lines 57% (4/7) large-cell NSCLC cell lines 6% (1/17) other NSCLC cell lines	Protein down-regulation in 100% (8/8) primary SCLC 5% (1/22) primary NSCLC	[54]
	ND	Protein down-regulation in 86% (23/28) neuroendocrine tumors including typical (5/5) and atypical (2/2) carcinoids, large cell neuroendocrine carcinoma (6/7) and SCLC (15/18) 1% (1/74) NSCLC	[55]
Skin	68% (28/41) microdissected primary basal cell carcinoma	Protein down-regulation in 71% (29/41) basal cell carcinoma	[38]
Squamous epithelia of the mucous surfaces	35% (32/92) primary oral SSC 50% (3/6) oral displasia	mRNA down-regulation in 100% (32/32) methylation-positive SCC Protein down-regulation in all methylation-positive SCC (n=36)	[46]
	56% (20/36) vulval SCC 47% (15/32) VIN	mRNA down-regulation in 72% (26/36) vulval SCC	[56]
	ND	Protein down-regulation in 18% (5/27) cervical adenocarcinoma 13% (2/15) cervical adenosquamous carcinoma	[44]
	ND	Protein down-regulation in 33 % (3/9) primary cervical carcinoma	[45]
Endometrium	Methylation associated with IHC-negativity (primary adenocarcinoma *n*=19)	Protein down-regulation in 43 % (20/46) primary adenocarcinoma	[45]
Ovary	79% (11/14) clear cell adenocarcinoma 26% (5/19) serous adenocarcinoma 36% (4/11) mucinous adenocarcinoma 20% (2/10) endometrioid adenocarcinoma	Protein down-regulation in 79% (11/14) clear cell type 10% (2/19) serous type 18% (2/11) mucinous type 10% (1/10) endometrioid type of adenocarcinoma	[35]
	58% (7/12) ovarian cancer cell lines 30% (3/10) IHC-positive 80% (8/10) IHC-negative microdissected adenocarcinoma	mRNA down-regulation in 58% (7/12) ovarian cancer cell lines Protein down-regulation in 26% (27/102) primary adenocarcinoma	[57]
	Methylation associated with IHC-negativity (primary adenocarcinoma *n*=17)	Protein down-regulation in 67% (14/21) primary adenocarcinoma	[45]
Prostate	ND	Protein down-regulation in 90% (n=111) primary carcinoma and PIN	[58]
	100% (41/41) microdissected primary prostate adenocarcinoma	Protein down-regulation in 63% (26/41) primary carcinoma and PIN	[33]
	Methylation associated with IHC-negativity (primary adenocarcinoma *n*=30)	Protein down-regulation in 45% (18/40) primary carcinoma	[45]
	33% (1/3) prostate carcinoma cell lines	Protein down-regulation in 94% (72/76) primary carcinoma	[59]

HCC = hepatocellular carcinoma; IHC = immunohistochemistry; *n* = number of analyzed cases; ND = not determined; NSCLC = non-small cell lung cancer; PIN = prostate intraepithelial hyperplasia; SCLC = small cell lung cancer; SSC = squamous cell carcinoma; VIN = vulval intraepithelial hyperplasia.

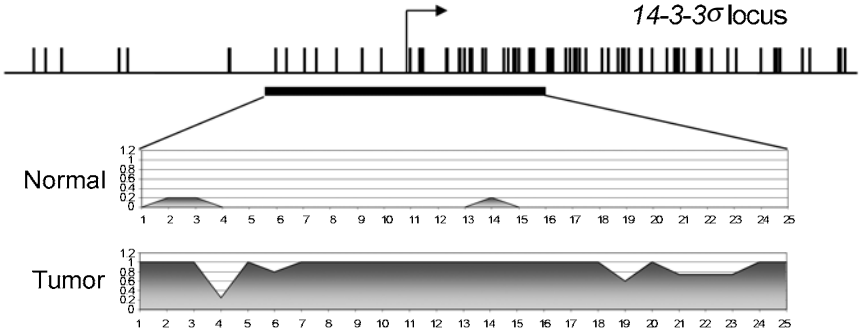

Fig. 3 The pattern of CpG methylation in promoter region of *14-3-3σ* in primary prostate epithelial cells ("Normal") and the prostate cancer cell line LNCaP ("Tumor"). The depicted areas correspond to 2.5 kb genomic DNA sequences. Vertical bars represent CpG-dinucleotides. The position of the transcription start site is indicated by an arrow. The black bar indicates the region studied by bisulfite sequencing. Grey shaded chart areas represent frequencies of methylated CpGs. The *y*-axis corresponds to the relative abundance of methylation of the CpG-dinucleotide at the indicated relative positions.

many cases silencing of *14-3-3σ* was identified by a candidate approach. We recently identified silencing of *14-3-3σ* when we applied an unbiased, genome-wide approach to identify genes silenced in prostate cancer cell lines (Fig. 3, [33, 34]). Alterations in the expression of 14-3-3σ were shown to be characteristic for distinct cell types in tumors of the same location. For example, 14-3-3σ expression was used to distinguish various types of ovarian carcinoma [35], and to discriminate between urothelial and prostate carcinoma [36].

In breast cancer progression the frequency of hypermethylation gradually increases upon transition from atypical hyperplasia to invasive breast carcinoma [37] (Tab. 1). Moreover, hypermethylation of *14-3-4σ* was detected in adjacent histologically normal breast epithelium, while CpG methylation in breast epithelial cells was never observed in individuals without evidence of breast cancer. It has been proposed that hypermethylation of *14-3-4σ* is an early event during breast cancer formation that precedes any morphological change in tissue architecture or cell shape [37]. We made a similar observation in tissues containing basal cell carcinoma (BCC) of the skin [38]. Morphologically benign epidermis, adjacent to BCC, showed the presence of CpG methylation, whereas the epidermis derived from a distant location was free of *14-3-3σ* methylation. Rare methylation in non-neoplastic cells from cancer patients was also observed in prostate [33] and hepatocellular carcinoma [39]. CpG methylation in normal, tumor-adjacent tissue is not an exclusive feature of the *14-3-3σ* gene, since it has also been shown for other genes silenced by CpG methylation, such as the $p16^{INK4A}$ [40], *RAR-β2* [41] and *TR-β1* [42].

Recently, the expression of 14-3-3σ protein in breast carcinoma was analyzed using a proteomic approach complemented by immunohistochemical analysis [43]. In contrast to several previous studies [32, 37, 44, 45], which showed loss of 14-3-3σ expression due to epigenetic silencing in the majority of analyzed samples, Moreira and colleagues could detect the loss of 14-3-3σ protein only in 9 of 105 primary breast carcinomas. This issue should therefore be examined in larger numbers of samples.

An inverse correlation between *p53* mutations and loss of *14-3-3σ* expression through promoter methylation has been reported for oral SCC [46]. This observation is consistent with a role of p53 as an upstream regulator of the *14-3-3σ* gene. However, in BCC and prostate cancer, mutations of *p53* are known to be late events, whereas silencing of *14-3-3σ* takes place early in tumor progression, e.g. in PIN lesions of the prostate [33, 38].

Presumably, CpG methylation is also involved in inactivation of *14-3-3σ* expression in non-epithelial cells during normal development. For example, its methylation was detected in stromal [33, 37, 38] and lymphoid cells [47], in which this gene is not expressed. Unexpectedly, a strong methylation of *14-3-3σ* can be detected in normal epithelial cells of the pancreatic gland, whereas pancreatic cancers display overexpression of 14-3-3σ associated with promoter hypomethylation [48-50].

FUNCTIONS OF THE 14-3-3σ PROTEIN

14-3-3σ, or stratifin (SFN), belongs to the 14-3-3 family comprising six other members in mammals (designated β, ε, γ, η, τ, ζ). 14-3-3 proteins form homo- and heterodimers and bind to protein ligands via a consensus binding motif which has to be phosphorylated on serine/threonine residues. The association with 14-3-3 proteins regulates the associated proteins by cytoplasmic sequestration, mask-

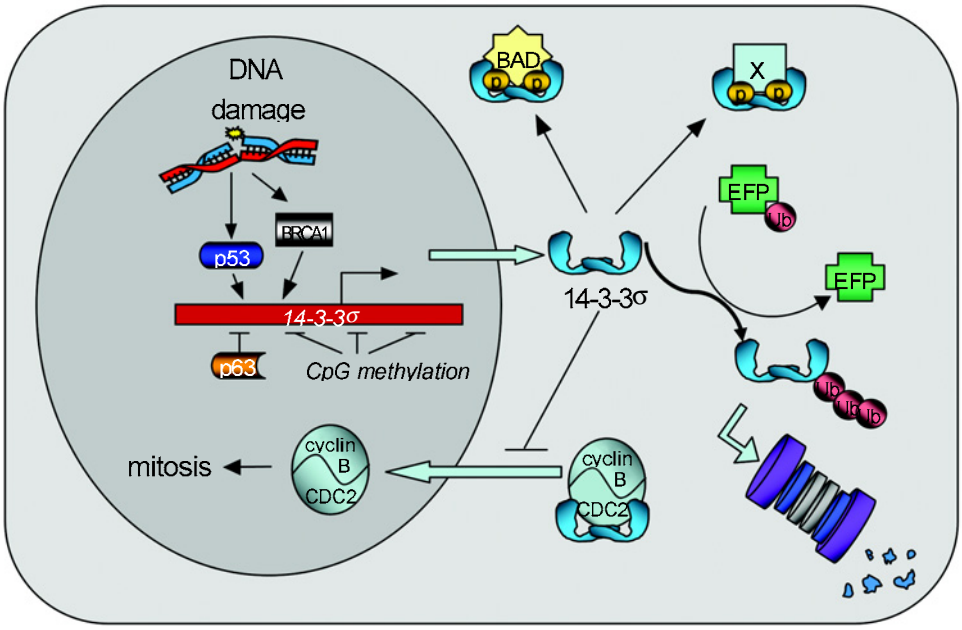

Fig. 4 Regulation and function of 14-3-3σ. After DNA damage p53 binds to the promoter and induces transcription of the *14-3-3σ* gene. BRCA1 acts as a co-activator in the induction of *14-3-3σ* transcription by p53. In the cytoplasm 14-3-3σ forms homodimers and binds to a variety of ligands phosphorylated at the serine/threonine residues in a consensus binding motif. The nuclear translocation of cyclin B/CDC2 complexes required for the initiation of mitosis is inhibited by 14-3-3σ. The BH3-domain protein BAD phosphorylated by AKT associates with 14-3-3σ and loses its ability to antagonize the function of the anti-apoptotic BCL2-like proteins. A dominant negative form of p63 (ΔNp63α) represses *14-3-3σ* expression. CpG methylation mediates epigenetic silencing of *14-3-3σ* in neoplasia and in normal, non-epithelial cells. A RING-finger-dependent E3 ubiquitin (Ub) ligase (EFP) targets 14-3-3σ for proteasomal degradation. X = unknown binding partner.

ing of interaction domains and export or import sequences, prevention of degradation, modulation of enzymatic activity and transactivation, and by facilitation of protein modifications (reviewed in [30, 60]).

14-3-3σ in the DNA damage response

14-3-3σ is the only 14-3-3 isoform, which is induced after DNA damage (Fig. 4). Induction of *14-3-3σ* mRNA by DNA damage was identified in a SAGE (serial analysis of gene expression; [61]) based screen for genes induced after γ-irradiation in colorectal cancer cells expressing wild type p53 [62]. In this study p53 was found to directly activate the transcription of *14-3-3σ* [62]. The tumor suppressor protein p53 is a nodal point in the network of DNA damage induced signaling pathways and mediates inhibition of cell proliferation, presumably to allow time for repair or to permanently arrest damaged cells [63]. The net effect of p53 activation, which is typically proliferation arrest or apoptosis, is determined by the subset of transcriptional targets activated in a certain cell type and signaling context. Together with $p21^{WAF1}$, *14-3-3σ* belongs to a subset of p53 targets which mediate cycle arrest, whereas other p53 target genes mediate programmed cell death. ΔNp63α, an isoform of the p53 homolog p63, which lacks a transactivation domain, was shown to bind to the p53-responsive element in the *14-3-3σ* promoter, but, in contrast to p53, represses transcription of *14-3-3σ*. Interestingly, 14-3-3σ was shown to participate in the nuclear export of ΔNp63α protein and thereby promote its proteosomal degradation after DNA damage in squamous cell carcinoma of the head and neck [64]. BRCA1 was shown to affect G_2/M progression by inducing expression of *14-3-3σ* [65].

The ectopic expression of 14-3-3σ in colorectal cancer cells and primary prostate epithelial cells leads to the initiation of a G_2 arrest [62]. A similar effect was observed in breast carcinoma cell line in which *14-3-3σ* is epigenetically silenced [32]. Disruption of the *14-3-3σ* gene by homologous recombination in colon cancer cells results in impaired G_2/M checkpoint after DNA damage: γ-irradiated *14-3-3σ* knockout cells are unable to maintain a G_2 arrest and eventually undergo mitotic catastrophe [66, 67]. Moreover, *14-3-3σ*-deficient cells show increased genomic instability, characterized by loss of telomeric repeat sequences, chromosome end-to-end fusions and non-

reciprocal translocations [68]. Breast cancer cells with hypermethylation of *14-3-3σ* display increased genomic instability [32]. Furthermore, RNAi-mediated down-regulation of 14-3-3σ protein expression in prostate carcinoma cells was shown to compromise the stability of a DNA damage induced G_2 arrest [33] and promote polyploidisation (Lodygin *et al*, unpublished results). Taken together, these results strongly argue for an important role of 14-3-3σ in the maintenance of genomic integrity after DNA damage and suggest that the epigenetic silencing of this gene in cancer cells may contribute to the chromosomal instability.

14-3-3σ functions in keratinocytes

Among epithelial tissues, the epidermis is characterized by the highest level of 14-3-3σ expression. Keratinocytes which exit from the stem cell compartment and differentiate show an increase in 14-3-3σ expression [69], suggesting that 14-3-3σ may be linked to terminal differentiation of epithelial cells. In addition, *14-3-3σ* was implicated in senescence of primary human keratinocytes cultured *in vitro*. Experimental inactivation of *14-3-3σ* in primary keratinocytes by an anti-sense approach allows keratinocytes to evade senescence and become immortal without additional genetic alterations [70]. How the loss of 14-3-3σ contributes to immortalization is not clear. However, it is possible that the epigenetic silencing of *14-3-3σ* in keratinocytes may contribute to BCC formation by inactivation of senescence pathways which normally limit the proliferative capacity.

One unexpected function of 14-3-3σ in keratinocytes was recently described by Ghahary and colleagues: differentiated keratinocytes secrete 14-3-3σ and thereby induce expression of collagenase/MMP-1 (matrix metalloproteinase 1) in co-cultured dermal fibroblasts [71, 72] (see comment: [73]). Supporting this finding the addition of the recombinant 14-3-3σ protein to fibroblast cultures had also induced MMP-1 expression. MMP-1 is a key enzyme for the degradation of collagen, which is the main component of the extracellular matrix (ECM) produced largely by fibroblasts. Excessive accumulation of ECM proteins during wound healing process can lead to the fibrosis and formation of hypertrophic posttraumatic scars. The delay in epithelialization, due to either infection or severity of injury, increases the frequency of developing fibrotic conditions. Therefore, 14-3-3σ is a candidate factor which could mediate the signaling from keratinocytes to the fibroblasts to switch from collagen accumulation to maturation and remodeling during wound healing. Moreover, the keratinocytes co-cultured with dermal fibroblasts seem to express a greater level of *14-3-3σ* mRNA, suggesting the existence of a stroma-derived factor which regulates *14-3-3σ* expression.

14-3-3σ protein ligands

Candidate approaches led to the identification of several important cellular targets of 14-3-3σ. For example, binding of 14-3-3σ to CDC2/cyclin B1 complex provides a mechanistic explanation for the effect of induced 14-3-3σ expression observed after DNA damage. Cytpoplasmic 14-3-3σ prevents nuclear localization of the CDC2/cyclin B complex, which is required for progression through mitosis [66] (Fig. 4).

In addition, 14-3-3σ was shown to bind to G_1-specific CDKs (cyclin dependent kinases), such as CDK2 and CDK4 [74]. Therefore, 14-3-3σ may contribute to the G_1 arrest in epithelial cells. 14-3-3σ contains a putative CDK-interaction motif found in other CDK-binding proteins [74]. Presumably the interaction between 14-3-3σ and CDKs is not dependent on ligand phosphorylation, and might therefore depend on the availability of the 14-3-3σ protein, which is regulated on the transcriptional and post-translational level.

14-3-3 proteins are known to antagonize programmed cell death. The molecular basis for this effect may by the interaction with the BH3-family protein BAD, which is sequentially phosphorylated by protein kinase B (AKT) and protein kinase A (PKA) in response to survival stimuli. Binding of the phosphorylated BAD protein to 14-3-3 releases the anti-apoptotic BCL2 protein from the BAD/BCL2 heterodimeric complex, resulting in the inhibition of apoptosis [75]. 14-3-3σ was shown to interact with BAD [76]. The pro-apoptotic BH3 protein BAX also associates with 14-3-3σ [77]. Loss of this interaction may contribute to the enhanced sensitivity of *14-3-3σ*-deficient HCT116 cells to DNA damage.

The levels of 14-3-3σ proteins seem to be tightly controlled by ubiquitin-mediated proteasomal degradation. Ubiquitylation of 14-3-3σ by the EFP protein, which is a oestrogen-regulated E3 ubiquitin ligase, targets 14-3-3σ for proteasomal degradation [78]. The functional significance of this regulation was illustrated by experiments in animal models, in which EFP status modulated the growth of breast cancer xenografts in a 14-3-3σ-dependent manner [79].

Recently, we applied a targeted proteomics approach to identify novel binding partners of 14-3-3σ [79]. Among 117 candidates identified by tandem affinity purification and subsequent mass-spectrometry-based MudPIT (multidimensional protein identification technology) analysis were 14 previously characterized and 103 new 14-3-3σ interacting proteins. The known function of the identified 14-3-3σ interacting proteins indicates that 14-3-3σ is involved in the regulation of cytoskeletal dynamics, polarity,

adhesion, mitogenic signaling and motility in addition to its previously identified inhibitory effect on cell cycle progression.

It is a conundrum how the induction or loss of 14-3-3σ has profound effects in the presence of large amounts of endogenous 14-3-3 proteins expressed by the 6 other 14-3-3 isoforms. Recently, we solved the crystal structure of the un-liganded 14-3-3σ dimer (PDB entry 1YZ5) and compared this structure to the known structure of 14-3-3τ and ζ (published in this issue; see reference [80]). Thereby, we could identify putative determinants of 14-3-3σ ligand binding and dimerization [80]. High structural conservation was identified in the phosphopeptide binding cleft among the 14-3-3 isoforms σ, τ and ζ. A region adjacent to this posphopeptide binding pocket and a non-conserved loop (amino acids 203-215) were identified as potential ligand binding specificity regions [80]. Furthermore, non-conserved residues at the dimerization interface were identified the 14-3-3σ structure [80]. These residues may determine the dimerization specificity. Such selective dimerization specificity may exist as we only detected 14-3-3σ homodimers and heterodimers with endogenous 14-3-3γ protein in a targeted proteomics analysis of 14-3-3σ [79].

CONCLUDING REMARKS

Down-regulation of 14-3-3σ expression is commonly found in different types of carcinoma, including early stages of tumor development. This indicates that the function of 14-3-3σ may contribute to the prevention of malignant transformation of epithelial cells. The known functional properties of 14-3-3σ suggest, that epigenetic silencing of *14-3-3σ* in cancer cells results in defective cell-cycle control after DNA damage and promotes genomic instability. Recently identified, novel 14-3-3σ-interacting proteins implicate 14-3-3σ in the regulation of diverse cellular processes (migration, adhesion, polarity, MAPK and wnt signaling). It will be interesting to determine to which extent 14-3-3σ loss by CpG methylation or p53 mutation contributes to deregulation of these processes in cancer cells. In the future, the frequent cancer-specific CpG methylation of *14-3-3σ* may be used as a diagnostic tool in combination with recently developed techniques that allow the analysis of the methylation status in disseminated cancer cells found in body fluids (for a review, see [81]).

ACKNOWLEDGEMENTS

Work in Heiko Hermeking's lab is supported by the Max-Planck-Society and the Dr- Mildred Scheel-Stiftung/ Deutsche Krebshilfe.

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