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Review Article

14-3-3σ, the double-edged sword of human cancers

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Abstract: 14-3-3σ is a member of a highly conserved family of 14-3-3 proteins that are present in all eukaryotic organisms. 14-3-3σ has been considered as a tumor suppressor with reduced expression in some human cancers while its increased expression causes resistance to anticancer agents and radiation that cause DNA damages. The increased expression of 14-3-3σ may also predict poor prognosis in some human cancers. Thus, 14-3-3σ may play an important role as a double-edged sword in human cancers, which may attribute to its property as a molecular chaperone by binding to various protein ligands important to many cellular processes such as cell cycle checkpoint regulation and apoptosis in response to DNA damages. In this article, we will review recent studies and progresses in understanding 14-3-3σ as a double-edged sword in human cancers.

Key words: 14-3-3σ, tumorigenesis, metastasis, prognosis, drug resistance, expression regulation

Introduction

14-3-3σ (also called stratifin) is a member of a highly conserved family of 14-3-3 proteins that are present in all eukaryotic organisms [1]. The human family of 14-3-3 proteins consists of 7 known highly conserved isoforms (α/β, γ, ε, ζ, η, σ, and θ/τ) (**Figure 1**), and these proteins are widely expressed in different human tissues [2-4]. They bind to phosphoserine proteins that have consensus motifs RSXpSXP and/or RXY/FXpSXP [2, 5-7]. They play important roles in many biological activities by directly binding to and altering the subcellular localization and/or stability of key molecules in various signaling cascades [7, 8]. 14-3-3σ was originally characterized as a human mammary epithelium marker 1 [9, 10] and was later rediscovered as an important molecule for cell cycle checkpoint regulation [11, 12]. More than one hundred ligands of 14-3-3σ have been identified and these ligands are involved in many important cellular processes such as apoptosis, cell proliferation and cycle regulations [4, 13]. In this article, we will review latest progresses in understanding the structure and function of 14-3-3σ, its regulation, and its role as a double-edged sword in human cancers.

Structure and function of 14-3-3σ

The primary amino acid sequences of all 7 human 14-3-3 proteins are highly conserved (**Figure 1A**). They share about 44.4% amino acid residues that are identical in all isoforms of human 14-3-3 proteins. As shown in the phylogenetic tree in **Figure 1B**, 14-3-3σ is a more recent member of the family and evolutionarily closer to 14-3-3β and more distant from other members of the family.

Crystal structures of all seven mammalian 14-3-3 isoforms have been solved [6, 13-16]. 14-3-3σ shares many structural features with the other members of the family. All 14-3-3 molecules exist as dimers and have an overall structure that resembles a flattened horseshoe (**Figure 2A**). Each subunit of a 14-3-3 protein is composed of nine antiparallel α-helices denoted as αA to αI. Residues on αA and αB from one subunit provide interactions with the opposing residues on αC and αD from the other subunit in a dimeric complex. This

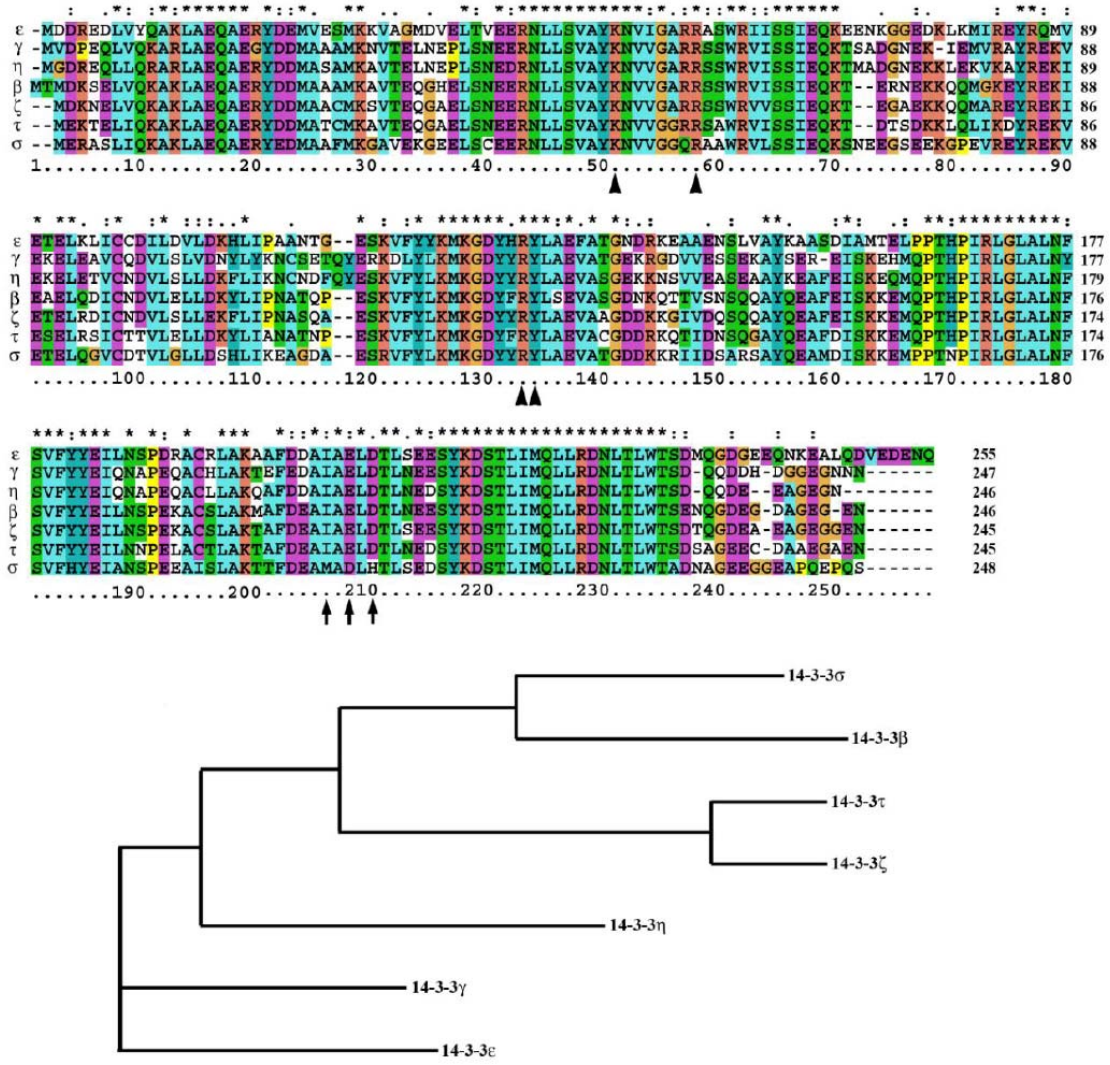

Figure 1. Sequence alignment and phylogenetics of human 14-3-3 proteins. The amino acid sequence of all 7 human 14-3-3 protein isoforms were aligned using the program Clustal X2 (http://www.clustal.org) and analyzed for phylogenetics using the programs TCoffee (http://tcoffee.vital-it.ch/cgi-bin/Tcoffee/tcoffee_cgi/index.cgi) and PhyloDraw (http://pearl.cs.pusan.ac.kr/phylodraw). Asterisks indicate identical amino acid residues that are present in all human 14-3-3 proteins while colon and semin colon indicate the partially conserved residues among all human 14-3-3 proteins. The arrowheads indicate the conserved residues that are involved in binding the phosphate group of phosphor-protein ligands. The arrows show three unique amino acid residues in 14-3-3σ that may help the protein in ligand binding selectivity and specificity for 14-3-3σ.

arrangement of the subunits creates a central channel that has a diameter of about 15Å with a 10Å depth. Conserved salt bridges are found in 14-3-3 dimer interfaces. The salt bridge between Arg^{18} in one subunit and Glu^{91} in another has been found in all 14-3-3 isoforms while the salt bridge between Asp^{21} and Lys^{87} exists in all except 14-3-3ε. In addition, conserved hydrophobic interactions involving residues Leu^{12}, Ala^{16}, Leu^{62}, Ile^{65} and Tyr^{84} are found in all 14-3-3 dimer interfaces.

In contrast to the above common features shared by all human 14-3-3 family members in their dimeric structures, the structural differences between 14-3-3σ and other isoforms shed lights on its uniqueness in function and behavior. 14-3-3σ differs from

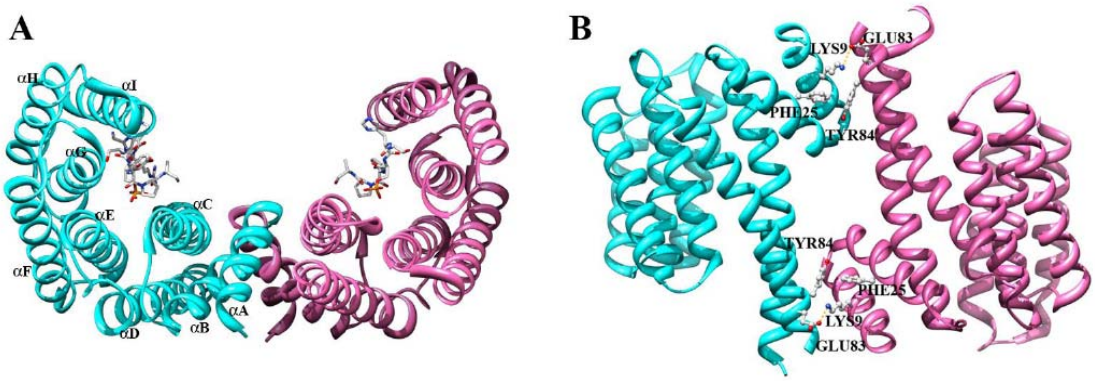

Figure 2. The complex structure of 14-3-3σ and its ligand phosphopeptide. A, the complex structure of 14-3-3σ shown in perpendicular to the two-fold symmetry (PDB ID: 1YWT). The dimeric structure of 14-3-3σ is shown as ribbon while the phosphopeptide ligand with a sequence of MARSHpSYPAKKK is shown as sticks. The two monomeric subunits are colored in cyan and pink, respectively, with the α helices indicated in one subunit. B, the dimeric structure shown in parallel to the two-fold symmetry. The dimeric structure of 14-3-3σ is shown as ribbon with two subunits colored differently. The 4 amino acid residues (Lys^{9}, Glu^{83}, Phe^{25}, and Tyr^{84}) that are involved in the dimeric interactions specifically for 14-3-3σ subunits are shown as balls and sticks. The salt bridges are indicated as dotted lines.

other isoforms except 14-3-3γ in that it prefers to form homo-dimers whereas other isoforms can form both homo- and hetero-dimers with other members of the 14-3-3 family [13]. Examination of the dimeric interface shows that two pairs of interactions may account for this distinctive property of 14-3-3σ (**Figure 2B**). The first one is the salt bridge between Lys^{9} and Glu^{83}, which occurs twice due to the 180^{o} symmetry and exists only in 14-3-3σ. The other is the ring-ring interaction between Phe^{25} and Tyr^{84}, which is also unique to 14-3-3σ and occurs twice [13]. Other residues, Ser^{5}, Glu^{20} and Glu^{80} in 14-3-3σ are also thought to stabilize the formation of homo-dimeric complex because mutations of these three residues promoted hetero-dimerization of 14-3-3σ with other 14-3-3 isoforms [17]. Mutations of the Phe^{25} and Gln^{55} residues of 14-3-3σ promoted little hetero-dimerization although they decreased the ability of 14-3-3σ to form homo-dimers [17]. Interestingly, the combined mutations of all five residues resulted in a mutant that could no longer form homo-dimer but could form hetero-dimers with all other six 14-3-3 isoforms.

One of the important questions regarding 14-3-3σ is whether the homo-dimerization is required for its function. To answer this question, the mutant 14-3-3σ carrying mutations for all five residues as described above (Ser^{5}, Glu^{20}, Glu^{80}, Phe^{25}, and Gln^{55}) were used to determine their effect on cell proliferation [17]. Unlike wild type 14-3-3σ, over-expression of the mutant 14-3-3σ did not inhibit the growth rate of the transfected cells [17]. While this functional assay of cell growth rate is not the best one for 14-3-3σ, the finding appears to indicate that homo-dimerization may be required for 14-3-3σ function. Furthermore, the potential structural changes induced by these mutations are not yet known. Thus, it is not clear if the mutation-generated loss of 14-3-3σ function is due to loss of homo-dimerzation or simply due to its structural changes. It also remains to be determined if mutations of these key residues to amino acids that are different from the ones already generated would cause similar losses in dimerization properties and 14-3-3σ functions.

As discussed above, two consensus motifs containing a phosphoserine have high affinity to all 7 14-3-3 isoforms, although the presence of neither motif is required for all 14-3-3 binding partners/ligands. Based on the structure of 14-3-3σ complexed with a peptide ligand that has the RSXpSXP motif, it was thought that the major interactions between 14-3-3σ and the ligand were the same as that found in 14-3-3ζ isoform [13]. The ligand with the RSXpSXP motif binds 14-3-3σ in a narrow

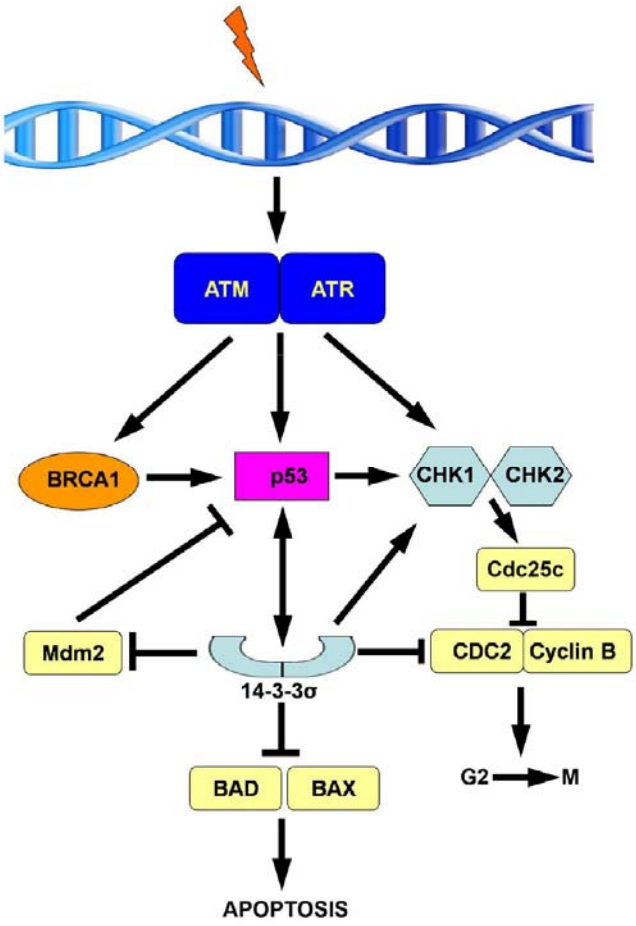

Figure 3. The functions of 14-3-3σ in DNA damage response. In DNA damage response, 14-3-3σ is up-regulated and plays roles in G2 arrest and apoptosis delay. Both BRCA1 and p53 up-regulate 14-3-3σ expression in response to DNA damages, which in turn up-regulates p53 via direct binding and by down-regulating MDM2 in a positive feed back loop. By binding to CDC2 and activating Chk1, 14-3-3σ induces G2 arrest in response to DNA damages and by binding to Bad and/or Bax, 14-3-3σ prevents DNA-damage-induced apoptosis.

basic groove composed of residues from αC, αE, αG and αI. Amino acid residues Lys[49], Arg[56], Arg[129], and Tyr[130] bind and stabilize the negatively charged phosphate group of the ligand (**Figure 1**). These conserved interactions are a consequence of strictly conserved key residues within the phosphopeptide-binding site. The selectivity of specific protein partners may involve other sites that are unique to 14-3-3 isoforms.

Interestingly, 14-3-3σ differs from other family members in the area opposite to the concave where the phosphopeptide binds. Three unique residues Met[202], Asp[204], and His[206] in this area of 14-3-3σ are replaced by Ile, Glu, and Asp, respectively, in all other 14-3-3 isoforms (**Figure 1**). These residues in 14-3-3σ may make more contacts with protein ligands and account for specificity and selectivity of protein partners. Indeed, mutations of these three residues of 14-3-3σ to the conserved Ile, Glu, and Asp of other family members changed the affinity of 14-3-3σ to Cdc25C [13].

It is also noteworthy that a short stretch of 10-20 amino acid residues at the carboxyl terminus of 14-3-3 proteins is the most divergent region in amino acid sequences of these proteins (**Figure 1**) and all crystal structures lack this domain because no electron density could be localized in x-ray structure [6, 13-16]. This domain consists of a stretch of acidic residues and may be highly flexible. Deletion of the carboxyl terminus of 14-3-3ζ generated a protein that has higher affinity to its protein ligands [18]. Furthermore, the binding-deficient mutant protein ligands could also bind to the mutant 14-3-3ζ lacking the carboxyl terminus. These observations suggest that the flexible carboxyl terminus of 14-3-3 proteins may play a role in regulating 14-3-3 protein binding to their ligands. However, similar studies with other 14-3-3 isoforms will be needed to confirm this conclusion.

Regulation of 14-3-3σ expression

The expression of 14-3-3σ may be regulated at multiple steps. Unlike other 14-3-3 proteins (ε, τ, β, η, and ζ), the expression of 14-3-3σ was increased following treatment with γ-irradiation or other DNA-damaging agents in a p53-dependent manner [11, 19]. In the promoter sequence of the 14-3-3σ gene, there is a p53-responsive element which may be responsible for its p53 dependence. It has also been found that 14-3-3σ mRNA level was down regulated in BRCA1$^{-/-}$ cell line and re-introduction of BRCA1 restored the mRNA level of 14-3-3σ [20]. Ectopic over-expression of BRCA1 in HCC1937 and MCF-7 cells also increased the expression of 14-3-3σ [21]. In addition, the BRCA1 induction of 14-3-3σ requires the presence of wild type p53, suggesting that BRCA1 may work through p53 on the p53 response element present in the 14-3-3σ promoter [20]. Therefore, 14-3-3σ expression is up-regulated in response to DNA damages possibly in a BRCA1 and p53 dependent manner (**Figure 3**).

14-3-3σ expression also appears to be regulated epigenetically. It has been shown that the 14-3-3σ gene is methylated in many tumors, suggesting that the expression of 14-3-3σ may be silenced by methylation in tumors, which may contribute to tumorigenesis (see below). The methylation of 14-3-3σ gene have been detected in human cancers of breast [22, 23], endometrium [24], head and neck [25, 26], liver [27], small cell lung cancer (SCLC) [28, 29], ovary [30], prostate [31], skin [32], stomach [33], and vulva [34, 35] (**Table 1**). In most of these studies, the decreased expression of 14-3-3σ in cancers has also been confirmed at mRNA or protein levels and consistent with the methylation status of 14-3-3σ gene in cancer tissues (see **Table 1**).

In the case of human lung cancers, two studies reported that the 14-3-3σ gene was methylated in SCLC but not in NSCLC (non small cell lung cancer) [28, 29]. The expression of 14-3-3σ also did not appear to change in the NSCLC, consistent with the finding on the lack of methylation of 14-3-3σ gene in these cancers. In another study, only 39 of 115 cases of NSCLC showed methylation of 14-3-3σ gene [36]. Thus, the frequency of 14-3-3σ gene methylation in NSCLC may be low compared to SCLC. However, the decreased expression of 14-3-3σ has been frequently observed in NSCLC cell lines [28, 37], suggesting that other mechanisms of regulation of 14-3-3σ gene expression may be involved.

Indeed, it is noteworthy that the methylation status of 14-3-3σ gene in some tumors does not correlate to the 14-3-3σ expression level. For example, high 14-3-3σ expression was found in 226 cases (75%) and low levels in 76 cases (25%) of vulva squamous cell carcinomas, although 14-3-3σ methylation was identified in 100% of the 57 cases tested [35]. It was also found in this study that the protein levels of 14-3-3σ did not correlate to its mRNA levels in the vulva squamous cell carcinomas tested. Interestingly, similar findings were also observed in uterine cervix [38] and colorectal cancers [39] where the mRNA and protein levels of 14-3-3σ did not correlate to each other. In the study of uterine cervix, it was found that the cancer tissues that were negative in immunostatining of 14-3-3σ protein were stained positive for 14-3-3σ mRNA in the in-situ hybridization assay [38]. These observations indicate that the expression of 14-3-3σ may also be regulated at post-transcriptional level, such as mRNA stability, translational efficiency, and protein stability. Indeed, it has been shown previously that the stability of 14-3-3σ protein is regulated by estrogen via ubiquitination and proteosome-mediated degradation [40].

Taken together, the expression of 14-3-3σ may be regulated at multiple levels (**Figure** 3). The 14-3-3σ gene may be silenced by hypermethylation in some tumor cells and become activated in response to DNA damages via BRCA1/p53-dependent transcription. The expression of 14-3-3σ may also be regulated post-transcriptionally by protein stability and degradation. However, the post-transcriptional regulations are understudied and more detailed analyses are needed to elucidate the mechanism of this level of regulation.

14-3-3σ and tumorigenesis

As shown in **Table 1**, 14-3-3σ expression has been found to be lost or decreased in many human cancers. including that of breast [41], endometrium [24], head and neck [25, 26], liver [27], SCLC [28, 29], prostate [31, 42], and vulva [34] (**Table** 1). In a proteomic profiling analysis of three different human lung cancer cell lines in comparison with normal human lung fibroblast and bronchial epithelial

Table 1. 14-3-3σ gene methylation, expression, and prognosis in human cancers

Cancer Sites	Gene Methylation (Positive / Total Cases)	Expression[a]		Prognosis	References
		mRNA	Protein		
Breast	Yes (99/110)				[22]
	Yes (15/18)				[23]
			Down		[41]
				Poor	[49]
			Down	Poor	[60]
Cervix		Up	Up		[38]
Colon		Up	Up		[50]
			Up	Poor	[39]
Endometrium	Yes (21/33)		Down		[24]
				Good	[61]
Head & Neck		Up			[51]
	Yes (32/92)	Down	Down		[25]
	Yes (16/16)		Down		[26]
Liver	Yes (17/19)		Down		[27]
Lung (SCLC)	Yes (6/13)		Down		[28]
	Yes (8/24)		Down		[29]
Lung (NSCLC)	No		No Change		[28]
	No		No Change		[29]
	Yes (39/115)			Poor	[36]
		Up	Up		[52]
Ovary	Yes (11/20)			Good	[30]
				No	[62]
Pancreas		Up	Up		[53]
		Up			[54]
		Up	Up		[55]
			Up	Poor	[56]
		Up			[85]
		Up			[43]
Prostate			Down		[42]
	Yes (41/41)		Down		[31]
Skin	Yes (28/41)		Down		[32]
Stomach	Yes (26/60)				[33]
		Up	Up		[50]
Vulva	Yes (19/36)	Down			[34]
	Yes (57/57)	Variable	Up	No	[35]

[a] The expression level shown was in comparison to normal tissues.

cells, 14-3-3σ was found to be one of the genes that were down-regulated in lung cancer cell lines [37]. Decreased expression of 14-3-3σ has also been found with many other cancer cell lines such as that of breast [22].

The finding that 14-3-3σ expression was reduced in many tumor cells led to a speculation that 14-3-3σ may be a potential tumor suppressor gene. Interestingly, the expression of other 14-3-3 isoforms (α/β, and ζ) was not changed in breast [41] and pancreatic [43] cancers, suggesting that the possible tumor suppressor role may be specific to 14-3-3σ. In the case of breast cancers, the lost expression due to hypermethylation occurred not only in primary tumors [22] but was also found in ductal carcinoma in situ and atypical hyperplasia [23], suggesting that hypermethylation silencing of 14-3-3σ gene may be an early event in mammary neoplastic transformation.

Furthermore, it was found that over-expressing 14-3-3σ inhibited the growth of Her2-transformed NIH-3T3 cells in nude mice, possibly by stabilizing p53 in these cells [44]. It was also found that over-expression of 14-3-3σ in nasopharyngeal carcinoma cell lines inhibited the anchorage-independent growth of these cells and reduced their tumor volume in nude mice [45]. However, direct evidence is still lacking in demonstrating that reducing 14-3-3σ expression would cause or contribute to tumorigenesis. In fact, many cancer cell lines express high levels of 14-3-3σ and grow similarly as cells that have lower levels of the protein [46, 47]. Loosing 14-3-3σ expression caused mitotic catastrophe under genotoxic stress [19] and the cells without 14-3-3σ appeared to have little growth advantage (see discussion on drug resistance below). Furthermore, disabling 14-3-3σ by generating a truncated protein lacking 40 amino acids at carboxyl terminus with a mutation led to defective ectodermal differentiation, cutaneous abnormality, and death at birth for homozygous mice [48]. Ectopic over-expression of wild type 14-3-3σ rescued the cutaneous abnormality of this animal model. No tumor incidence with this animal model expressing a truncated 14-3-3σ has yet been reported.

It is also noteworthy that the lost expression of 14-3-3σ in breast cancers may be a sporadic event and that its expression is up-regulated in some breast tumors [49]. It appears that a majority of breast cancer cells with basal/myoepithelial phenotype have 14-3-3σ expression whereas some of the breast cancer cells with luminal epithelial cell differentiation have decreased or lost 14-3-3σ expression. Increased 14-3-3σ expression (mRNA and/or protein) has also been found in cancers of colon [39, 50], head and neck [51], NSCLC [52], pancreas [43, 53-56], and stomach [50] (**Table 1**). Clearly, the role of 14-3-3σ as a tumor suppressor gene based on the findings of its reduced expression in some cancers is questionable and more work is certainly needed to investigate its possible role in tumorigenesis.

14-3-3σ and cancer metastasis and invasion

Recently, it has been suggested that 14-3-3σ may play an important role in cancer metastasis and invasion. Higher 14-3-3σ expression has been found to correlate significantly with large tumor size and deeper invasion of vulva squamous cell carcinoma [35]. Using invasion and wound healing assays, Neupane et al. showed that ectopically over-expressing 14-3-3σ in pancreatic cancer Panc-1 cells increased the EGF-stimulated invasion and motility [43]. Most recently, Li et al. identified 14-3-3σ as one of the proteins that are related to lymph node metastasis of lung squamous cell carcinoma using a proteomic analysis and laser capture dissection [57]. They further demonstrated that reducing 14-3-3σ expression by siRNA silencing increased in-vitro invasive ability of HTB-182 and A549 cells while enforced expression of ectopic 14-3-3σ decreased these abilities.

Furthermore, it has been reported previously that 14-3-3σ is secreted as a keratinocyte-derived collagenase-stimulating factor, which could increase the expression of collagenase [58, 59]. Secretion of 14-3-3σ has also been observed with pancreatic cancer cells [43]. These findings are intriguing since 14-3-3σ appears to lack known signal sequences for secretion. Nevertheless, the role of 14-3-3σ in stimulating collagenase expression has been shown by addition of recombinant 14-3-3σ to culture medium of dermal fibroblasts [58, 59]. Thus, it is possible that 14-3-3σ expression may play an important role in invasion and motility following its secretion.

However, it was also observed that over-expression of ectopic 14-3-3σ in DLD1-tTA cells inhibited cellular migration in a wound healing assay [4] whereas knocking-down 14-3-3σ expression in T3M4 cells had no effect on migration [43]. Clearly, it may be too early to conclude that 14-3-3σ plays any role in tumor metastasis and invasion and more work are needed to investigate if the role of 14-3-3σ in migration, metastasis, and invasion is cell type dependent and on the mechanism of 14-3-3σ secretion and stimulation of collagenase expression. A study with an animal model of metastasis will be ideal to help address these issues.

14-3-3σ and cancer prognosis

Despite the findings in many studies that breast cancer and some other cancers have lower level of 14-3-3σ than that of normal tissues (**Table 1**), it was found that patients with breast carcinomas (both luminal or

basal/myoepithelial phenotypes) who have higher cytoplasmic staining of 14-3-3σ also have shorter survival compared with patients who have lower 14-3-3σ staining [49]. When the tumors that are positive with estrogen receptor were examined, the positive relationship between 14-3-3σ expression and poor prognosis was even more significant. This later finding is very interesting because it has been shown that estrogen regulates the stability of 14-3-3σ by affecting its proteosome-mediated degradation [40]. In another study of breast carcinoma, it was shown that the expression of 14-3-3σ, together with cyclin B1, had positive correlations with and predicted poor prognosis [60].

Similar to the findings in breast cancers, 14-3-3σ expression appears to correlate with poor prognosis also in other cancers (**Table 1**). Recently, Perathoner *et al.* found that 14-3-3σ expression was an independent prognosis marker for poor survival of colorectal cancer patients and that the higher level of 14-3-3σ was associated with the higher grade colorectal cancers [39]. In a study of pancreatic cancers, it was also found that 14-3-3σ expression was a marker for poor prognosis [56]. However, in the later study, the correlation of 14-3-3σ expression with poor prognosis had no statistical significance, indicating that role of 14-3-3σ in predicting prognosis of pancreatic cancers may be more complex than that of colon cancers. In a study of NSCLC, Ramirez *et al.* [36] reported that the methylation status of 14-3-3σ gene correlated with prognosis. The median survival of 22 methylation-positive patients was significantly longer than the 29 methylation-negative ones. However, these authors did not determine the correlation between 14-3-3σ expression and survival. Because the methylation status of 14-3-3σ gene does not necessarily correlate with its expression level (see discussion above), the prediction of outcome using methylation may not be accurate. Nevertheless, the above studies strongly suggest that the tumors with higher expression level or less methylation of the 14-3-3σ gene may respond poorly to therapy in a clinical setting in human cancers of breast, colon, lung, and pancreas. It is also possible that chemotherapy selects the resistant cancer cells with higher expression levels of 14-3-3σ, which then causes recurrence of the disease and eventual failure of anticancer therapy.

In prostate cancers, it was reported that 14-3-3σ expression increased as tumor progresses and its expression was lower in cancers compared to normal tissues [42]. Adenocarcinomas with high Gleason scores (>7) had significantly higher staining intensities and higher percentages of 14-3-3σ immunoreactive cells than adenocarcinomas with low Gleason scores (<7). Adenocarcinomas with lymph node metastases had higher percentages of 14-3-3σ expression compared with adenocarcinomas without metastases. Furthermore, androgen-independent prostate cancer cell lines appear to express higher levels of 14-3-3σ than the androgen-dependent cells [46]. Similar results were also observed with endometrial cancers [24]. While 14-3-3σ expression is lower in histological low grade endometrial cancers compared to normal tissues due to hypermethylation, its expression increased in high grade tumors due to hypomethylation of the gene. These findings indicate that 14-3-3σ may play some role for the poor response to treatment of later stage cancers [46].

Despite the above findings that 14-3-3σ expression correlates with and may predict poor prognosis of breast, colorectal, prostate, and pancreatic cancers, opposite observations have also been made in other cancers including endometrium [61] and ovary [30], where it was found that the absence or low expression level of 14-3-3σ predicted poor survival or showed no correlation (see **Table 1**). In another study of 192 cases of ovarian cancer patients, no significant correlation between 14-3-3σ expression and survival was found [62]. Thus, it is possible that 14-3-3σ in prognosis prediction is cancer type dependent, and more detailed studies on this issue will be needed.

14-3-3σ and drug resistance

The findings that 14-3-3σ expression may predict poor prognosis suggest that it may contribute to resistance to anticancer therapeutics. Recently, a proteomic profiling analysis of Adriamycin-selected multidrug resistant breast cancer cell line MCF7/AdVp3000 in comparison with its parental drug sensitive MCF7 cell line showed that 14-3-3σ is one of the genes that are up-regulated in the drug resistant cells [47]. Interestingly, the expression level of 14-3-3σ also positively

correlates with the resistance level of a series of MCF7 cell lines of intermediate resistance that were generated during the selection of MCF7/AdVp3000 cells. Knocking down 14-3-3σ expression in the drug-resistant MCF7/AdVp3000 cell reduced its resistance to Adriamycin and mitoxantrone while ectopic over-expression of 14-3-3σ in the parental drug sensitive MCF7 cell increased its resistance to these drugs [47]. Furthermore, ectopic over-expression of 14-3-3σ in prostate cancer cell line DU145 increased the resistance to Adriamycin and mitoxantrone in these cells and knocking down 14-3-3σ expression in LNCaP and CWR22 cells decreased resistance to these drugs [46]. Thus, the elevated expression of 14-3-3σ likely causes drug resistance of these cells.

Similar proteomic analyses of drug-selected pancreatic cancer cell line, EPP85-181RNOV [63], as well breast cancer cell line, MCF7/Adr [64, 65] also showed that 14-3-3σ expression is up-regulated in these drug resistant cells. Ectopic over-expression of 14-3-3σ in a pancreatic cancer cell line PaCa-2 indeed increased its resistance to Adriamycin, mitoxantrone, γ-irradiation, as well as gemcitabine (Li et al., unpublished observations). Over-expressing 14-3-3σ in the pancreatic cancer cell line Panc-1 also increased survival rate of this cell line in response to cisplatin treatment while knocking down 14-3-3σ in T3M4 cells sensitized cisplatin-induced apoptosis [43]. Thus, 14-3-3σ likely plays an important role in resistance to DNA-damaging treatments in various cancer cell lines.

Taken together, 14-3-3σ likely contributes to resistance to DNA-damaging anticancer drugs and, thus, poor prognosis in cancer treatment. Because 14-3-3σ binds to various target proteins and may involve in many cellular processes, it is difficult to investigate the molecular mechanism of resistance induced by 14-3-3σ expression. However, recent progresses have shed some lights on the role of 14-3-3σ in regulating cell cycle check point and apoptosis in response to DNA damages, which may mediate the function of 14-3-3σ in therapeutic resistance and poor prognosis of human cancers.

Role in cell cycle regulation. One of the early studies showed that ectopic over-expression of 14-3-3σ alone without DNA-damaging treatment in wild type HCT116 cells induced G2 arrest [11]. However, this observation has not been reported in later studies. Instead, many of the later studies showed that the presence of 14-3-3σ helped cells arrest in G2 upon DNA-damaging treatments. Using somatic knock-out of 14-3-3σ in human colon cancer cell line HCT116, it was found that the cells without 14-3-3σ failed to arrest in G2 in response to DNA-damaging drug treatment [19]. Instead, the cells went through a process called mitotic catastrophe and death. These cells were also defective in G2/M checkpoint regulation in response to γ-irradiation [66]. Similarly, it was later found that human breast cancer cell lines with 14-3-3σ deficiency had significantly less G2 arrest following γ-irradiation compared with the 14-3-3σ positive breast cancer cell lines [22]. An adenoid cystic carcinoma cell line (ACCY) that lack 14-3-3σ was also defective in G2 arrest following γ-irradiation [26]. Re-introduction of 14-3-3σ into the 14-3-3σ deficient cells helped these cells to arrest in G2/M phases following treatment with γ-irradiation [22, 26]. Most recently, it was shown that simply knocking down 14-3-3σ expression in prostate cancer DU145 cells also led to defective G2/M checkpoint regulation and cell death following mitoxantrone treatment [46]. Thus, 14-3-3σ likely plays an important role in G2/M checkpoint regulation in response to DNA-damages.

The mechanism of 14-3-3σ action in G2/M checkpoint regulation has been documented. One possible mechanism is that 14-3-3σ binds to the phosphorylated cdc2, a cyclin-dependent kinase, and arrests cdc2-cyclin B1 complex in cytoplasm following DNA damages, which in turn arrest cells in G2/M phase [12, 19, 46] (also see **Figure 3**). In a study of clinical breast cancer samples, the cytoplasmic staining of cyclin B1 level also correlated with the level of 14-3-3σ [60]. Dephosphorylation of cdc2-cyclin B1 complex followed by translocation from cytoplasm to nuclei is a key step to initiate mitosis [67-70]. The inability of cdc2-cyclin B1 to translocate into nucleus due to its phosphorylation and 14-3-3σ binding inhibits cell cycle progression, which allows cells to repair their DNA damages and maintain their genomic stability [71, 72]. Silencing 14-3-3σ expression removes this important regulator and causes cells to progress uncontrollably following DNA

damages, which results in accumulation of DNA damages and eventual mitotic catastrophe and cell death [19].

Chk1 is an important upstream kinase involved in G2/M checkpoint regulation in response to DNA damages [73]. In response to DNA damage, Chk1 is activated by ATM and/or ATR, which then phosphorylates and inactivates Cdc25c for G2 arrest (**Figure 3**). By comparing phosphorylation status of Chk1 in wild type and 14-3-3σ deficient HCT116 cells in response to radiation, Tian *et al.* showed that the radiation-induced Chk1 phosphorylation was associated with its binding to p53, p21 and 14-3-3σ [74]. Thus, the contribution of 14-3-3σ to G2/M checkpoint regulation may also involve the interaction between 14-3-3σ and Chk1. However, it is unknown how the binding of 14-3-3σ to Chk1 regulates Chk1 activation. Further studies are clearly needed to understand the molecular mechanism of action of 14-3-3σ in G2/M checkpoint regulation. It will also be interesting to determine if 14-3-3σ regulates the activation and function of Chk2, another upstream kinase important for G2/M checkpoint regulation in response to DNA damages.

Role in DNA-damage-induced apoptosis. In addition to the role in cell cycle checkpoint regulation in response to DNA damages, 14-3-3σ also contributes to resistance to drug-induced apoptosis via binding to and arresting pro-apoptotic factors (**Figure 3**). It has clearly been shown that the DNA damage-induced apoptosis was reduced in the presence of 14-3-3σ but increased by knocking down 14-3-3σ expression [43, 46, 66, 75].

Extrinsic and intrinsic pathways are two well-known apoptosis pathways. Unlike extrinsic pathway, which is activated by binding of ligands to death receptors, intrinsic pathway is activated by cellular stress, such as DNA damages [76, 77]. In response to extensive DNA damages and activation of sensor proteins in the intrinsic pathway, Bad and/or Bax, members of the Bcl-2 pro-apoptotic protein family, are translocated from cytoplasm to mitochondria, a key step for cytochrome C release from mitochondria to cytoplasm, followed by activation of caspases 9 and 3 and apoptosis. It has been shown that 14-3-3σ could bind to Bax in response to Adriamycin treatment and inhibit Bax translocation from cytoplasm to mitochondria in HCT116 cells, which in turn delays cytochrome C release from mitochondria and inhibits the apoptotic signal transduction [75]. Interestingly, Bad was not found to interact with 14-3-3σ in this study. However, using in-vitro pull down assay as well as two-yeast hybrid assay, Subramanian *et al.* [78] showed that 14-3-3σ as well as all other 14-3-3 isoforms could bind to Bad and inhibit apoptosis induced by co-expression of Bad and its activator Akt in COS-7 and HeLa cells. These observations suggest that 14-3-3σ and other 14-3-3 isoforms not only can bind to Bad and the binding may require its phosphorylation by Akt. Thus, 14-3-3σ may also contribute to resistance to DNA damage treatments by causing resistance to DNA-damage-induced apoptosis via binding to Bax and/or Bad (**Figure 3**). However, it is noteworthy that the binding of 14-3-3σ to Bax and Bad may be cell type specific. As mentioned above, the binding of 14-3-3σ to Bad was not detected in HCT116 cells [75] while this binding was found in HeLa and Cos7 cells [78]. It has also been reported that 14-3-3σ could not bind to Bad or Bax in pancreatic cancer cell lines T3M4 and Colo-357 [55]. The reason for these differences is currently unknown. It is, however, possible that the binding is dependent on phosphorylation of Bax or Bad and some cells may lack the pathway to phosphorylate these proteins.

It has been known that 14-3-3σ regulates the expression of p53 and MDM2 in response to DNA damage (see below). Thus, it is also possible that 14-3-3σ may up-regulate anti-apoptotic and down-regulate pro-apoptotic protein expression which in turn causes resistance to DNA-damage-induced apoptosis. Very recently, we found that ectopic over-expression of 14-3-3σ in pancreatic cancer cell lines up-regulated Bcl-2 expression (unpublished observation). Thus, 14-3-3σ may contribute to resistance to DNA-damage-induced apoptosis in multiple ways and in pancreatic cancers 14-3-3σ may cause resistance to DNA damages via up-regulating anti-apoptotic proteins and by-passing inhibition of pro-apoptotic proteins.

Role in regulating p53 and MDM2 stability. As discussed above, the expression of 14-3-3σ is regulated by p53, a tumor suppresser protein that is also a key protein in DNA damage response and plays an essential role in

transcription, cell cycle checkpoint regulation, DNA repair and apoptosis [79, 80]. Recently, Yang *et al.* [44] reported that 14-3-3σ could directly bind to and stabilize p53 with a positive feedback regulation. The domain in 14-3-3σ that is responsible for p53 binding appears to be located in the carboxyl terminus corresponding to the ligand-binding site.

The protein level of p53 is known to be mainly regulated by MDM2 in a feedback loop [79, 81-83]. Recently, it was also found that the carboxyl terminus of 14-3-3σ could directly bind to and destabilize MDM2 by accelerating MDM2 self ubiquitination and degradation [84]. This effect of 14-3-3σ on MDM2 antagonized the negative regulation of p53 by MDM2 and, thus, prolonged the half life of p53. Therefore, the expression of 14-3-3σ can stabilize p53 both by directly binding to p53 and by accelerating the turnover rate of MDM2, the p53 negative regulator, which in turn leads to regulation of cell cycle checkpoint and apoptosis in response to DNA damages (**Figure 3**).

Conclusion and perspectives

In conclusion, 14-3-3σ is clearly an important molecule that may play roles in tumorigenesis, prognosis, and resistance to DNA-damaging treatments by affecting multiple pathways including cell cycle checkpoint regulation and apoptosis. It may work as a tumor suppressor and its decreased expression may contribute to tumorigenesis in some tissues. However, its increased expression in tumors will cause resistance to anticancer agents and radiation that cause DNA-damages. Thus, 14-3-3σ potentially functions as a doubled-edged sword to contribute to tumorigenesis and resistance in cancer therapy. The function as a double-edged sword appears to attribute to the molecular chaperone property of 14-3-3σ by its ability to bind to a variety of important protein ligands at their consensus sites. 14-3-3σ may serve as a marker for diagnosis and prognosis of human cancers and a potential target for therapeutic developments in combinational therapy of cancers.

While the role of 14-3-3σ in resistance to DNA-damaging treatments have been demonstrated using cancer cell lines, the direct cause-effect relationship between 14-3-3σ expression and clinical prognosis has not been demonstrated. In fact, the past correlative studies on 14-3-3σ expression and prognosis are not consistent. While some studies suggested that 14-3-3σ expression predicts poor prognosis, others did not. It is possible that the direct contribution of 14-3-3σ to poor prognosis may be tumor type specific. This issue is particularly intriguing when considering the expression of 14-3-3σ in normal tissues. Some tumors such as pancreatic cancers appear to have higher expression compared to normal tissue controls while studies of other tumors such as prostate cancers have opposite findings. This variation in 14-3-3σ expression in different tumors also raises a concern regarding the tumor suppressor role of 14-3-3σ, which may also be tissue specific. Nevertheless, future vigorous studies with large populations of various cancers will be needed to further investigate the role of 14-3-3σ in tumor suppression and prognosis. Animal models will also be needed to address these issues. Another interesting research direction is the preliminary finding that 14-3-3σ may play some roles in cell migration and cancer metastasis. More studies including use of animal models are certainly needed to verify this finding and to understand the mechanism of 14-3-3σ function in cell migration and metastasis.

At the molecular level, a single study using mutations suggested that the homo-dimeric 14-3-3σ may be needed for its activity. More vigorous studies are needed to investigate the dimerization, the critical residues responsible for specific interactions, and the role of homo-dimerization in 14-3-3σ functions. Understanding these aspects of the protein is essential for us to design specific inhibitors of 14-3-3σ that can disrupt the dimerization and, thus, inhibit its function. Considering that all members of the human 14-3-3 protein family share the common ligand binding site in their structure and similar protein ligands, targeting the dimerization interface of 14-3-3σ, which is unique among the members of the human 14-3-3 family, may be the only ideal choice for discovering novel specific inhibitors of 14-3-3σ.

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