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REVIEW

The interplay between p66Shc, reactive oxygen species and cancer cell metabolism

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ABSTRACT

The adaptor protein p66Shc links membrane receptors to intracellular signalling pathways and has the potential to respond to energy status changes and regulate mitogenic signalling. Initially reported to mediate growth signals in normal and cancer cells, p66Shc has also been recognized as a pro-apoptotic protein involved in the cellular response to oxidative stress. Moreover, it is a key element in processes such as cancer cell proliferation, tumor progression, metastasis and metabolic reprogramming. Recent findings on the role of p66Shc in the above-mentioned processes have been obtained through the use of various tumor cell types, including prostate, breast, ovarian, lung, colon, skin and thyroid cancer cells. Interestingly, the impact of p66Shc on the proliferation rate was mainly observed in prostate tumors, while its impact on metastasis was mainly found in breast cancers. In this review, we summarize the current knowledge about the possible roles of p66Shc in different cancers.

Keywords Cancer, cancer metabolism, p66Shc, reactive oxygen species.

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The role of p66Shc in the cellular response to oxidative stress

p66Shc has been extensively studied for many years, mostly in the contexts of the cellular response to oxidative stress and the mammalian lifespan. Now, p66Shc is considered to be involved in many other biological and pathological processes. p66Shc belongs to the ShcA family of adaptor proteins that consists of three members: p46Shc, p52Shc and p66Shc, with molecular masses of 46, 52 and 66 kDa, respectively. In humans, all ShcA proteins are encoded by the same gene, localized at chromosome 1q21 [1–3]. p66Shc, p52Shc and p46 have similar domain structures and contain three functionally identical domains (the PTB–CH1–SH2 signature): a N-terminal phosphotyrosine-binding domain (PTB), a central proline-rich domain (CH1) and a carboxy terminal Src homology 2 (SH2) domain. In response to growth factor stimuli, p66Shc is phosphorylated at the same tyrosine residues (Y293, Y240 and Y317) as the other two ShcA members (p52Shc and p46Shc) [4,5] but exerts opposite effects, acting as a negative regulator of cell proliferation [6–9]. Interestingly, p66Shc differs from p52Shc and p46Shc by the presence of an additional N-terminal proline-rich collagen homology domain (CH2), which contains a serine phosphorylation site (Ser36) that is critical for its pro-oxidant properties. Moreover, p66Shc contains a functional region (CCB, a cytochrome c–binding region) within the CH2–PTB domains that is responsible for the interaction of p66Shc with cytochrome c [10]. The intracellular localization of p66Shc remains controversial. Several reports have indicated that this cytosolic adaptor protein exhibits different subcellular localizations. At the plasma membrane, p66Shc is involved in signal transduction that mediates receptor tyrosine kinase signalling (Ras/MAPK signalling), promotes Rac1 activation and triggers NADPH membrane oxidase reactive oxygen species (ROS) production. p66Shc has also been found in mitochondria-associated membranes (MAMs) and plasma membrane-associated membranes (PAMs) [11,12]. In the nucleus, p66Shc can inhibit FOXO transcription factors and thus regulate the expression of ROS-scavenging enzymes [13]. Moreover, p66Shc has been found in the mitochondrial intermembrane space (IMS), where it interacts with cytochrome c and takes part in ROS production [10]. In a classical model, the cellular response to oxidative stress (UV radiation or H_2O_2 treatment) is associated with p66Shc phosphorylation at Ser36 by PKCbeta, which leads to

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p66Shc prolyl isomerization by Pin-1 and dephosphorylation by PP2A; finally, p66Shc moves from the cytosol to the MAM fraction [12]. Moreover, the work of Giorgio *et al.* [10] indicates that p66Shc can be also translocated to the IMS through the TIM/TOM mitochondrial import machinery. At the MAM, p66Shc interacts with a currently unidentified protein. In turn, p66Shc translocates to the IMS, where its redox-active region (present at the N-terminal domain) is involved in the transfer of electrons from reduced cytochrome c to molecular oxygen and, finally, in mitochondrial H_2O_2 production [10]. This elevated mitochondrial H_2O_2 production increases intracellular H_2O_2 levels, which activates PKCbeta (phosphorylating p66Shc) and, finally, leads to the activation of a self-triggered loop. All of this indicates that p66Shc seems to be an important player in stress-induced apoptosis and ROS-induced ROS production.

p66Shc – the protein with many functions

Apart from the situation described above, p66Shc has also been implicated in cancer development, progression and metastasis. Depending on the damage intensity and the repair system efficiency, mutations that accumulate over time can trigger either cell death or tumorigenesis. p66Shc plays an important role in the elimination of damaged cells via ROS-induced apoptosis. A subtle balance exists between the effects of p66Shc-generated oxidative stress; oxidative stress can protect the tissue from the uncontrolled cancer cell expansion by eliminating these cells; however, when unrestrained, this stress can induce cellular damage and trigger tumorigenesis. Interestingly, no difference in spontaneous tumorigenesis was observed in p66ShcKO mice of both SV129 and BL6 strains after either UV or 7,12-Dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate (DMBA/TPA) exposure [14], confirming the first observation from 1999 that the ablation of p66Shc has no negative impact on the health of the animals [15]. Moreover, knocking out p66Shc abolishes the oncogenic effect of the absence of p53 and increases the survival of p66Shc/p53 double knockout animals [14]. Interestingly, the impact of p66Shc and p53 on superoxide dismutase (SOD2) downregulation may be important in cancer development, as decreased antioxidant defence system function leads to oxidative stress induction [16]. The loss of p66Shc expression leads to constant Ras activation and unregulated cellular proliferation, which is, for example, responsible for lung cancer development and metastasis [17]. p66Shc levels can be epigenetically regulated (inhibited) through CpG site methylation [18]. The oxidative stress response is regulated by the stress-activated transcription factor Nrf2, which has been found to bind to the p66Shc promoter to activate its transcription in normal epithelial cells. However, in lung cancer cells, the Nrf2 binding site at the p66Shc promoter is methylated; this blocks Nrf2 binding and p66Shc transcription. Lower levels of p66Shc may be responsible not only for metastasis (due to the inhibition of anoikis) but also for drug resistance (due to the higher Nrf2 levels) [19]. Interestingly, the histone deacetylase sirtuin-1 (SIRT-1) involved in ageing and age-related pathologies also has been found to regulate p66Shc expression by decreasing acetylation of histone H3 bound to the p66Shc promoter region. In fact, SIRT-1 inhibition or knockdown increases expression of p66Shc. Such regulation has been previously described in the case of hyperglycaemia-induced endothelial dysfunction [20]. However, taking into consideration the fact that SIRT1 can be involved in cancer promotion as well as in cancer suppression (depending on the tissue), the relationship between cancer type, p66Shc level and effect of p66Shc expression regulators is very complex. More information about the role of sirtuins (especially SIRT1) in several types of cancer can be found in [21] (Table 1).

Does the level of p66Shc depend on the cancer type?

Although p66Shc functions as a negative regulator of proliferation by preventing Grb-2 from binding and activating Ras and downregulating the activities of MAP kinases, p66Shc levels are found to be increased in many tissue specimens from patients with cancer.

A growing number of publications indicate that the levels of p66Shc are increased in many cancer cell lines; however, it is still under debate whether p66Shc up- or downregulation leads to cancer. Interestingly, in contrast to other ShcA isoforms (p46Shc and p52Shc, positive regulators of Ras), only p66Shc was found to be upregulated in highly metastatic variants of the human breast cancer cell line MDA-MB231 [22]. In another type of breast cancer cell line, overexpression of the HER2/neu proto-oncogene that encodes a transmembrane receptor tyrosine kinase correlated with undetectable p66Shc protein levels. No correlation was observed in the cases of human ovarian, lung and oral cancer cells, suggesting a highly cell-specific p66Shc expression mode [23]. Almost 11 years ago, decreased p66Shc levels and upregulated tyrosine phosphorylation of ShcA proteins were proposed as good markers for the diagnosis and prognosis of breast cancer [24,25] and IIA colon cancer [26].

p66Shc and steroid hormones

Several lines of evidence suggest that steroids might be involved in the early stages of carcinogenesis. As they can regulate p66Shc expression, sex hormones were described by Lee *et al.* as perfect candidates for carcinogenesis, with regard to their involvement in many diverse functions, including growth, proliferation and differentiation. A positive correlation has been demonstrated between p66Shc protein levels and

Table 1 p66Shc in cancers

		p66Shc		
Type of tumor	**ROS**	**Tyr phosphorylation**	**Ser36 phosphorylation**	**Literature**
Lung cancer	No data	*Decreased expression of p66Shc in lung cancer cell lines (H1155, H82, Fx293, H1466, H1975)*		[19,30]
		No data	Taxol treatment induces phosphorylation of p66Shc at Ser36	
Colon cancer	Elevated level of endogenous ROS. Decreased ROS in the case of low p66Shc level	*Overexpression of p66Shc*		[36,46]
		No data	No data	
Melanoma	Decreased level of H_2O_2	*MIA as a novel binding partner of p66Shc*		[35]
		No data	MIA protein produced by melanoma cells inhibits phosphorylation of p66Shc at Ser36 what decreases H_2O_2 level	
Breast cancer	Increased level of H_2O_2	*Increased expression of p66Shc in metastatic type of breast cancers*		[22,46]
		MDA-MB-231 cells exert strongly phosphorylation of p46 and p52	No data	
Ovarian cancer	Increased level of H_2O_2 in estrogens (E_2) treated cells	*Increased level of p66Shc in comparison with noncancerous cells*		[28,46]
		No data	No data	
Prostate cancer	Steroid hormones increase level of ROS	*Elevated level of p66Shc, which correlates with a growth rate of prostate cancer cells*		[27,30–32,46,47]
		Tyrosine phosphorylation of p66Shc not detected in different passages of LNCaP cells Increased phosphorylation of p52 at Y317 residues	Androgen – stimulated proliferation of prostate cancer cells correlates with decreased phosphorylation at Ser36	
Thyroid cancer	Increased level of H_2O_2	*TSH increases p66Shc expression both in vivo and in vitro*		[29,46]
		TSH pre-treatment significantly potentiated tyrosine phosphorylation of p66Shc induced by IGF-I	TSH pre-treatment causes insulin-induced p66Shc Ser36 phosphorylation	

human breast [22,25], ovarian [28], thyroid [29] and prostate [30] cancer cell proliferation rates. Veeramani *et al.* also indicated the requirement for ROS production in the "p66Shc-stimulated" proliferation of tumor cells. Therefore, p66Shc levels are significantly higher in steroid-dependent cancer types, in particular [30]. Additionally, elevated p66Shc protein levels were correlated with higher androgen receptor (AR) expression levels [27]. It should also be noted that p66Shc Ser36 phosphorylation, which is decreased by androgen/estrogen stimulation in cancer cells, can serve as a protective mechanism against cell death [30,31]. In turn, in breast cancers, higher p66shc levels were associated with tumors with higher metastatic capacities [22,31]. Curiously, steroid hormones have no effect on the other members of the Shc family of proteins, including p52Shc and p46Shc [22,32].

Prostate cancer

Steroid hormones have an impact on the function of cells through direct transcriptional regulation of proliferation, growth, differentiation and survival. They also contribute to the stabilization of p66Shc by inhibiting p66Shc ubiquitination in prostate and ovarian cancers [33]. Fast-proliferating prostate cancer cell lines express more p66Shc than slow-growing lines; however, when transfected with prostatic acid phosphatase (cPAcP), these fast growing lines tend to decrease p66Shc expression. On the contrary, slow-growing lines treated with EGF or 5α-dihydrotestosterone (DHT) began to proliferate faster and express more p66Shc [30]. Androgenic stimulation induces p66Shc expression and promotes oxidative stress, which can be abolished by antioxidant treatment. DHT additionally facilitates p66Shc pro-oxidant action by mediating p66Shc translocation to the mitochondria and promoting its interaction with cyt. c [31]. However, p66Shc knockdown did not affect prostate specific antigen (PSA) expression, suggesting that the p66Shc pathway might be involved in early androgenic cell proliferation, independently from androgen receptor-dependent gene expression [31].

Chronic lymphocytic leukaemia

Similarly to that in prostate cancer, the role of p66Shc has been studied in B cells in context of chronic lymphocytic leukaemia (CLL) [34]. CLL, characterized by a progressive accumulation of monoclonal CD5+ B cells in the peripheral blood, bone marrow and peripheral lymphoid organs, seems to be a good example of a lack of proper ROS-induced apoptotic signalling, which is often correlated with low p66Shc levels. Interestingly, the lower p66Shc levels observed in CLL B cells are not a result of p66Shc promoter methylation. Thus, the decreased p66Shc levels in CLL B cells could be caused by, for example, a p66Shc gene defect [34].

Malignant melanoma cancers

The unique regulatory mechanisms controlling p66Shc expression have been investigated by Kasuno *et al.* in malignant melanomas. They identified the melanoma inhibitory activity (MIA) protein, secreted by melanoma cells, as a novel binding partner of p66Shc and, simultaneously, an antagonist of Ser36 phosphorylation [35]. This protein takes part in melanoma progression and metastasis and likely inhibits p66Shc Ser36 phosphorylation by binding to the CH_2 domain of p66Shc. Moreover, Ser36 phosphorylation can also be suppressed in different manners, for example, by inhibiting JNK activity [35].

Other cancers

Colorectal carcinoma cells (RKO) have been characterized to exhibit increased p66Shc levels and hydrogen peroxide production, which lead to mitochondrial fragmentation and cell death, *in vitro*. Increased p66Shc levels have also been found in colon cancers [36], while decreased p66Shc protein levels have been found in human lung cancers [19,37]. Similarly, Abdollahi *et al.* [38] observed decreased p66Shc expression in malignant ovarian surface epithelial cells compared with control cells. In the case of gastric cancer, significantly increased p66Shc expression was found, but no correlation between the level and the stage of disease progression was observed.

p66Shc and cancer cell metabolism

Recent studies by Soliman *et al.* indicate the role of p66Shc in metabolic shifts in tumor cells. Their studies have suggested that p66Shc exerts a suppressive effect on cancer cell metabolism by dampening growth factor signalling. p66Shc silencing improves glucose uptake, enhances glycolytic metabolisms and redirects glucose-derived carbon into anabolic metabolism through actions mediated by mammalian target of rapamycin (mTOR) and S6 kinase (S6K) – a major downstream effector of mTOR [39,40]. The metabolic shift towards a more glycolytic metabolic state, which is frequently observed in highly proliferative cells, reduces mitochondrial ROS production and provides metabolic intermediates that sustain biosynthetic pathways. This observation supports the fact that p66Shc acts as an mTOR inhibitor and mediates feedback inhibition of cell growth and glucose metabolism [39,41]. Furthermore, p66Shc was proposed to contribute to mTOR/S6K activation in response to glucose, amino acids and insulin, indicating that p66Shc could be a sensor of nutrient abundance, mechanistically linked with ageing. In fact, p66Shc may act as an adaptor between ROS and mTOR by promoting insulin and nutrient signalling to S6K; this is demonstrated by the fact that p66Shc upregulation as well as its increased phosphorylation at Ser36 activates S6K [41].

Thus, it is currently accepted that p66Shc can switch the energy balance depending on the specific bioenergetic context to maintain proper ATP levels. For example, in human lung adenocarcinoma A549 cells, nutrient deprivation induces p66Shc expression, while p66Shc downregulation mitigates low-nutrient-induced autophagy [37]. Generally speaking, p66Shc suppresses anabolic metabolism and causes cell metabolism reprogramming towards glucose catabolism and oxidative respiration in tumors [39].

The hypothesis that p66Shc translates nutrient availability into mitochondrial ROS production has important implications in cancer and stem cell biology. In highly proliferative cells, increased oxidative stress might contribute to the elevated p66Shc levels, which could be involved in carcinogenesis [30]. Although p66Shc was initially reported as a pro-apoptotic molecule, cancer cells and cancer stem cells, which are adapted to survive in a metabolically stressed, nutrient-deficient microenvironment, often prefer glycolysis. These cells express aberrant levels of p53 and p66Shc, which are usually correlated with cell proliferation, high metastatic potential and poor prognosis [31,42]. Therefore, as is the case with other redox-dependent signal transduction pathways, p66Shc might play a pivotal role in coordinating survival autophagy, autophagic cell death and apoptosis, depending on the bioenergetic status.

Cancer cells and pluripotent cells often have hyperpolarized mitochondria that might result from decreased mitochondrial ATP production under normoxia and confer resistance to intrinsic apoptosis by inhibiting mitochondrial membrane permeabilization [43]. At the same time, p66Shc has displayed potential roles in carcinogenesis and stem cell self-renewal [14,44], thereby facilitating the acquisition of resistance phenotypes in these cells.

p66Shc and anticancer strategies

Metastasis, which in most cases is the event that leads to the deteriorated state of patients with cancer, represents one of the most difficult problems to overcome in anticancer strategies. Thus, many laboratories are trying to understand p66Shc involvement in metastasis. It is well established that p66Shc is important in anoikis, cellular death associated with the loss of anchorage. This mechanism was found to be impaired when cancer cells begin to migrate and colonize other tissues. The above-mentioned MIA protein, which interacts with p66Shc, has an impact on cellular adhesion, the p66Shc Ser36 phosphorylation pathway and pro-oxidant sensitivity. MIA overexpression in cancer cells significantly decreases the ability of cancer cells to undergo apoptosis by the mitochondrial and/or ROS-dependent pathways. Moreover, decreased sensitivity to oxidative stress can explain the unsuccessful treatment of patients, for example, patients with melanoma [35]. Interestingly, another transcription factor, a lymphocyte lineage-restricted transcription factor (Aiolos), has been found to interfere with the pro-oxidant function of p66Shc. Aiolos, which is frequently expressed in lung cancers, reconfigures the chromatin structure within the SHC1 gene, thereby silencing the p66Shc anchorage reporter and blocking anoikis, both *in vitro* and *in vivo*. In lung cancer tissues and single cells, p66Shc expression is inversely correlated with Aiolos expression [45,46,47]. All of these observations indicate that future potential cancer therapies acting via p66Shc regulation can include treatments that upregulate its ubiquitination pathway, affect the MIA–p66Shc interaction and alter p66Shc promoter methylation.

Concluding remarks

Our understanding of the involvement of p66Shc in ROS production and the impact of p66Shc on cancer metabolism remains insufficient and requires additional systematic research. Further investigation will permit a better understanding of the involvement of p66Shc not only in cancers but also in other pathologies accompanied by intracellular oxidative stress.

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Conflict of interest

The authors declare no conflict of interests.

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