A novel role of Shc adaptor proteins in steroid hormone-regulated cancers

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Abstract

Tyrosine phosphorylation plays a critical role in growth regulation, and its aberrant regulation can be involved in carcinogenesis. The association of Shc (Src homolog and collagen homolog) adaptor protein family members in tyrosine phosphorylation signaling pathway is well recognized. Shc adaptor proteins transmit activated tyrosine phosphorylation signaling that suggest their plausible role in growth regulation including carcinogenesis and metastasis. In parallel, by sharing a similar mechanism of carcinogenesis, the steroids are involved in the early stage of carcinogenesis as well as the regulation of cancer progression and metastatic processes. Recent evidence indicates a cross-talk between tyrosine phosphorylation signaling and steroid hormone action in epithelial cells, including prostate and breast cancer cells. Therefore, the members of Shc proteins may function as mediators between tyrosine phosphorylation and steroid signaling in steroid-regulated cell proliferation and carcinogenesis. In this communication, we discuss the novel roles of Shc proteins, specifically p52Shc and p66Shc, in steroid hormone-regulated cancers and a novel molecular mechanism by which redox signaling induced by p66Shc mediates steroid action via a non-genomic pathway. The p66Shc protein may serve as an effective biomarker for predicting cancer prognosis as well as a useful target for treatment.

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Introduction

Cancers in steroid hormone-responsive tissues presently account for more than 35% in men and more than 40% in women of all newly diagnosed cancers in the United States (Henderson & Feigelson 2000). Extensive research has clearly demonstrated that the abnormal changes in the levels, frequencies, and types of steroid hormones are important contributors to the development of major cancer types such as the cancers of the prostate (androgen, estrogen), testes (in utero estrogen), breast (estrogen, progesterone), ovary (FSH, estrogen, androgen), uterine endometrium (estrogen), and thyroid (TSH, estrogen; Henderson & Feigelson 2000). Thus, many studies have been focused on the involvement of steroids in the regulation of tumor development, cancer cell proliferation, progression, and metastatic processes. It is now evident that either in the early stage of carcinogenesis or in the advanced metastatic phenotype, steroid hormone action goes far beyond the classical receptor-mediated gene regulation. These steroid hormone-related cancers may share common mechanisms of carcinogenesis, such as DNA damage/mutation as well as the elevated levels of various growth factors induced by the excess of steroids, leading to aberrant growth regulation (Dickson & Lippman 1987, Dabrosin et al. 1997, Devanesan et al. 2001). Recent advances further indicate that these hormones could also induce rapid, non-genomic responses and a convoluted network of interactions with different intracellular signaling pathways.
Non-genomic actions by steroid hormones

Numerous studies have demonstrated the non-genomic action of steroid hormones, including androgens and estrogens, in cellular processes such as cell proliferation and motility (Berridge et al. 1998). The most intriguing facts on the non-genomic nature of steroids are that the effects depend on their rapid response and insisting no direct binding of nuclear receptors to gene expression, i.e., seconds to minutes and their insensitivity to the inhibitors of transcription and translation and/or the antagonists of the classical intracellular steroid hormone receptors. The non-genomic effects of steroids could be mediated by multiple pathways, and are discussed briefly as follows.

Direct and acute action of steroids

Steroids may have direct action on target molecules independent of steroid receptors. One such effect, for example, is the direct binding and activation of protein kinase C (PKC) isoforms such as PKC\(_\alpha\) and PKC\(_\beta\) by aldosterone and 17β-estradiol (E\(_2\)) respectively via binding directly to their regulatory domains C\(_2\) that mediates calcium (Ca\(^{2+}\)) binding and results in the autophosphorylation of these kinases (Alzamora & Harvey 2008). Furthermore, aldosterone and E\(_2\) rapidly and directly stimulate phospholipase A\(_2\) (PLA\(_2\)) and cyclooxygenase (COX), which also result in the rapid increase in intracellular [Ca\(^{2+}\)]\(_i\) (Harvey et al. 2002). The other direct and acute effects of steroids are their rapid action on voltage-gated and calcium-activated ion channels. E\(_2\) activates the calcium-activated potassium channels via binding directly to its regulatory β-subunit (Valverde et al. 1999). Testosterone rapidly activates ATP-sensitive K\(^+\) channels (K\(_{ATP}\)) via opening K\(_{ATP}\) channels (Er et al. 2004) and inhibits the L-type and T-type calcium channels (I\(_{Ca,L}\); Michels et al. 2006, Er et al. 2007).

Rapid action of steroids involving classical intracellular steroid receptors

Steroids may bind to the classical intracellular steroid receptors and activate the second messenger pathways such as c-Src kinase that rapidly stimulate the MAPK/ERK and PI3K/AKT kinase pathways (Migliaccio et al. 2000). Interestingly, an androgen receptor (AR)/Src/modulator of non-genomic action of estrogen receptor (MARN) complex and the cooperative association of c-Src, estrogen receptors (ERs), and AR activates MAPK and c-Src kinase pathways respectively (Kousteni et al. 2001, Unni et al. 2004). Estrogens on binding to ER\(_{\alpha}\) may also serve as a transcriptional co-activator activating several transcriptional factors, such as activator protein 1 (AP-1), nuclear factor kappa B (NF-κB), and SP-1 in a non-genomic manner (Ray et al. 1997, Jakacka et al. 2001, Safe 2001). Steroids can also activate cAMP-dependent protein kinase A (PKA) via the transmembrane sex hormone-binding globulin (SHBG) receptor in association with transmembrane G-protein-coupled receptor (GPCR; Fortunati 1999, Rosner et al. 1999). The activation of PKA via the induction of cAMP by SHBG is observed in both prostate and breast cancer cells (Fortunati et al. 1996, Nakhla et al. 1997).

Rapid action of steroids involving non-classical membrane-bound steroid receptors

Steroids may undergo non-genomic action by binding to distinct non-classical membrane-bound steroid receptors. Several reports have presumed the presence of androgen- and estrogen-binding sites in a number of cells (Benten et al. 1999a,b, Armen & Gay 2000, Kampa et al. 2002). Interestingly, both the membrane androgen receptor (mAR) and the membrane ER (mER) are found to be associated with an integral membrane protein caveolin that facilitates the assembling of several signaling molecules, including phosphatidylinositol 3-kinase (PI3K), Ras, and Src kinase in their scaffold domain (Okamoto et al. 1998, Kim et al. 1999, Lu et al. 2001). Furthermore, mER perhaps exists as in a cytoplasmic pool and the rapid action requires their interaction with caveolin in association with MNAR, Shc and growth factor receptors, and striatin that translocated ER to the plasma membrane (Wong et al. 2002, Lu et al. 2004, Song et al. 2005).

Rapid action of membrane steroid receptors involving GPCR

The most preserved non-genomic action of steroid hormones is the rapid increase in intracellular calcium concentration [Ca\(^{2+}\)] mediated via GPCR that constitutes α-, β-, and γ-subunits (Lieberherr & Grosse 1994, Benten et al. 1998), which ultimately results in the rapid activation of MAPK/ERK and PI3K/AKT pathways, leading to the activation of PKC and PKA (Kelly et al. 1999, Estrada et al. 2003). The interaction of mAR with GPCR results in the dissociation of Gα-subunit and the signal is transmitted from Gβγ through the activation of effector molecules including c-Src, Raf, and phospholipase C (PLC; Pierce et al. 2002). GPCR itself may also serve as the membrane receptor, i.e., binding of E\(_2\) to an orphan GPCR, termed...
GPR30, plays a critical role in the rapid signaling of E2-mediated stimulation of Ras-dependent MAPK activation through the phosphorylation of Shc (Luttrell et al. 1996).

Rapid action of membrane steroid receptors via trans-activation of growth factor receptors
The rapid non-genomic actions of membrane steroid receptors may function via trans-activation of the growth factor receptors (Levin 2005), and of all membrane steroid receptors, mER is the paramount and well studied. The phenomenon is further confirmed by the co-existence of endogenous membrane receptors, including AR and ER, G-proteins, GPCR, growth factor receptors (EGFR, IGFR), non-receptor tyrosine kinases (Src, Ras), and linker proteins such as MNAR and striatin in the plasma membrane termed as ‘signalosomes’ (Hammes & Levin 2007). Alternatively, steroids may activate growth factor receptor kinase activity by inhibiting the regulatory phosphatases (Meng et al. 2000).

Rapid non-transcriptional action of membrane steroid receptors
The other non-genomic action of steroids involving membrane receptors is the non-transcriptional effects of these receptors that provoke the posttranslational modifications including phosphorylation. By regulating kinases and phosphatases, steroids influence the cell functions such as cell motility via modifying actin cytoskeleton (Kampa et al. 2002, Meyer & Feldman 2002, Levin 2005).

Rapid action of steroids on membrane fluidity
Steroids may mediate the non-genomic fashion through changes in membrane flexibility. Androgens via interacting with phospholipids in the lipid bilayer decrease the membrane fluidity, and subsequently alter the function of Na\(^{+}/K\)^+ and Ca\(^{2+}\) ATPase systems and also influence cellular adhesion and cell–cell interaction (Duval et al. 1983, Van Bömmel et al. 1987).

In summary, we propose that though the non-genomic actions of steroids are mediated through multiple pathways, both genomic and non-genomic effects are interlinked as non-genomic actions of steroids ultimately influencing at least one of the classical genomic-mediated transcriptional activities. Nevertheless, the molecular mechanisms of steroids, especially androgens and estrogens, in both the development and progression of human endocrine-related cancers at the non-genomic levels need further investigations. Along with these observations, in this review, we emphasize a novel non-genomic action of steroids promoting various stages of carcinogenesis via Shc proteins.

Cross-talk of tyrosine phosphorylation signaling and steroids in carcinogenesis
Every step of carcinogenesis is essentially controlled by various growth factors and their receptors, either it is steroid regulated or not. Growth factors, including nerve growth factor (Engebraaten et al. 1993, Sachs et al. 1996), fibroblast growth factor (Mignatti et al. 1991, Engebraaten et al. 1993, Taylor et al. 1993), platelet-derived growth factor (Engebraaten et al. 1993, Choudhury et al. 1997), epidermal growth factor (EGF; Engebraaten et al. 1993, Hamada et al. 1995), keratin growth factor (Sachs et al. 1996), hepatocyte growth factor (Pelicci et al. 1995, Sachs et al. 1996), interleukin 2 (Ratner et al. 1992), insulin, and insulin-like growth factors (Stracke et al. 1989) are known to be involved in regulating cell proliferation, motility, invasion, and/or migration of various cell types. The activated receptors triggered by growth factors, cytokines, or adhesion molecules facilitate the docking of Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domain-containing adaptor molecules that transduce signals via downstream intracellular cascades. Each of the receptors for the ligands described above activates the c-Src homology and collagen homolog (Shc) adapter proteins for signal transduction, which suggests a conceivable role of the Shc proteins in various stages of carcinogenesis.

Recently, several lines of evidence indicate that Shc proteins mediate diverse biological activities; for example, they may mediate steroid actions other than serving as adaptors in tyrosine phosphorylation signaling. Since the role of Shc proteins in mediating tyrosine phosphorylation signaling and in regulating oxidative stress-induced apoptosis has received much attention (Migliaccio et al. 1997, 1999, Ravichandran 2001), in this communication, we will first briefly overview Shc proteins and then focus our efforts on discussing the novel roles of Shc proteins, specifically p52\(^{\text{Shc}}\) and p66\(^{\text{Shc}}\), in steroid-regulated cancers.

Members of Shc family: structure and function in tyrosine phosphorylation signaling
Molecular structure of Shc isoforms
Shc proteins were first cloned using an SH2-coding sequence as a probe, and the Shc family includes three
isoforms with molecular masses of 46, 52 and 66 kDa, which are encoded by the same gene at chromosome 1q21 (Pelicci et al. 1992). A promoter in the first intron of Shc locus transcribes the mRNA of p66\textsuperscript{Shc}, whereas an alternate promoter and splicing generates the other two Shc isoforms, i.e., p46\textsuperscript{Shc} and p52\textsuperscript{Shc} (Ventura et al. 2002). These three isoforms of Shc protein contain overlapping amino acid sequences that contribute to a SH2 domain at the COOH-terminal and a PTB domain at the NH\textsubscript{2}-terminal, separated by a central region enriched in proline and glycine residues, i.e., collagen homology (CH1) domain (Ravichandran 2001), as shown in Fig. 1. The SH2 domain (\textasciitilde 100 amino acids) is the prototype for protein–protein interaction modules that mediate the formation of multiprotein complexes during signaling (Pawson & Scott 1997, Yoshida et al. 2004). Structurally, p66\textsuperscript{Shc} differs from p52\textsuperscript{Shc} and p46\textsuperscript{Shc} by virtue of its unique NH\textsubscript{2}-terminal, a 110-amino acid CH\textsubscript{2} region, which is also rich in proline and glycine residues (Migliaccio et al. 1997).

**Subcellular localization of Shc isoforms**

p66\textsuperscript{Shc} is expressed primarily in epithelial cells, while p52\textsuperscript{Shc} and p46\textsuperscript{Shc} are expressed ubiquitously (Migliaccio et al. 1997). Most of p66\textsuperscript{Shc} protein is distributed throughout the cytosol and a fraction of p66\textsuperscript{Shc} localizes within the inner membrane and intermembrane spaces of mitochondria (Orsini et al. 2004, Ventura et al. 2004, Giorgio et al. 2005, Nemoto et al. 2006). p46\textsuperscript{Shc} is found to be localized in the mitochondrial matrix (Orsini et al. 2004, Ventura et al. 2004, Nemoto et al. 2006). Unlike p46\textsuperscript{Shc} and p66\textsuperscript{Shc}, p52\textsuperscript{Shc} is translocated to the plasma membrane from cytosol upon stimulation by growth factors, e.g., EGF (Migliaccio et al. 1997).

**Figure 1** A schematic organization of Shc isoforms. The Shc proteins include three isoforms that are encoded by the same gene. They contain overlapping sequences. Three major tyrosine phosphorylation sites have been identified within the CH1 domain in all Shc isoforms. The unique CH2 domain of p66\textsuperscript{Shc} isoform consists of 110 amino acids and contains a serine phosphorylation site (Ser-36). The PTB domain of p46\textsuperscript{Shc} is deficient of the first 46 amino acids.

**Shc isoforms in tyrosine phosphorylation signaling**

**p46\textsuperscript{Shc}**

Shc proteins are expressed in distinct patterns and exhibit diverse biological functions. Ventura et al. (2004) have reported specifically p46\textsuperscript{Shc} as the first example to be localized in the mitochondrial matrix by means of a mitochondrion-targeting signal, which is inactive in p52\textsuperscript{Shc} and p66\textsuperscript{Shc}. Thus, p46\textsuperscript{Shc} may play a role in the signal transduction pathways regulating mitochondrial physiology (Ventura et al. 2004). In addition to mediating tyrosine phosphorylation signaling (McGlade et al. 1992, Migliaccio et al. 1997), p46\textsuperscript{Shc} may also modulate steroid action since steroids can regulate mitochondrial enzymatic activities (Ripple et al. 1997, 1999). Nevertheless, due to the limited studies on p46\textsuperscript{Shc} in steroid action in carcinogenesis, it will not be discussed further in this communication.

**p52\textsuperscript{Shc}**

p52\textsuperscript{Shc} is responsible for transducing anchorage-dependent growth signaling (Pelicci et al. 1992). In general, when cells are stimulated by growth factors, p52\textsuperscript{Shc} is recruited and binds to tyrosine kinase receptors through its PTB or SH2 domain, leading to its phosphorylation at tyrosine residues 239, 240, and 317 within the CH1 domain (Fig. 1; Rozakis-Adcock et al. 1992, van der Geer et al. 1996, Gotoh et al. 1996). Upon tyrosine phosphorylation, p52\textsuperscript{Shc} recruits Grb2/SOS through a binding event between the SH2 domain of Grb2 and Shc phosphotyrosine residues (Pelicci et al. 1992, Rozakis-Adcock et al. 1992), which ultimately results in the activation of Ras and the MAPK cascade for mitogenesis (Fig. 2; Bonfini et al. 1996). The PTB domain of p52\textsuperscript{Shc} binds to the phosphorylated tyrosine residues of receptor protein tyrosine kinases and functions similar to the SH2 domain. The CH1 and CH2 domains are putative SH3-binding regions (Lotti et al. 1996). Studies have also suggested that p52\textsuperscript{Shc} mediates steroid action on cell proliferation as well as cell survival via tyrosine phosphorylation signal pathway (Kousteni et al. 2001, Lee et al. 2004a). Its aberrant expression and activation may lead to the dysregulation of one of the multi-pathways in carcinogenesis by steroids, a topic of focus in this review.

**p66\textsuperscript{Shc}**

p66\textsuperscript{Shc} is also phosphorylated at its tyrosine residues as p52\textsuperscript{Shc} and p46\textsuperscript{Shc} upon growth factor stimulation, e.g., EGF treatment, and forms complexes with Grb2.
However, there are certain functional differences between p66Shc and the other two Shc members. Unlike p52Shc, p66Shc is unable to transform NIH3T3 mouse fibroblasts in culture (Migliaccio et al. 1999) and it could not augment EGF-induced extracellular signal-regulated kinases/mitogen-activated protein kinases (ERK/MAPK) activation in cell cultures such as HeLa, CHO, and COS-1 cells. One possible explanation is that increased expression of p66Shc has resulted in an elevated level of the basal activity of ERK/MAPK in the absence of stimulus, which thus minimizes the extent of further activation by growth factors (Migliaccio et al. 1997, Okada et al. 1997, Veeramani et al. 2005b). Recently, the task of p66Shc in mediating stress-induced apoptosis has received much attention (Migliaccio et al. 1999, Orsini et al. 2004, Giorgio et al. 2005). Once p66Shc is phosphorylated at Ser-36 in its CH2 domain in response to various stress factors, such as H$_2$O$_2$, UV radiation, and chemicals, e.g., Taxol, a fraction of cytosolic p66Shc associates with heat-shock proteins to mediate apoptotic response (Fig. 3; Orsini et al. 2004) and serves as an apoptotic sensitizer to those signals (Migliaccio et al. 1999). p66Shc also acts as a negative regulator of human and mouse T-cell survival and proliferation (Pacini et al. 2004). In parallel, p66Shc knockout mice exhibit a prolonged life span by 30% and those mouse embryo fibroblast (MEF) cells have increased resistance to oxidative and hypoxic stress (Migliaccio et al. 1999, Trinei et al. 2002, Zaccagnini et al. 2004). Thus, p66Shc may function as a longevity gene in mammals. Interestingly, p66Shc expression level in human dermal fibroblasts increases with aging, opposite to the knockout mouse model (Pandolfi et al. 2005). Collectively, these data indicate that p66Shc could function as a sensor and transduce signals in response to cellular stress while more studies are required for its role in human longevity.

Several lines of evidence suggest that aberrant expression of p66Shc could be involved in various stages of carcinogenesis (Jackson et al. 2000, Luzi et al. 2000, Ravichandran 2001, Davol et al. 2003, Lee et al. 2004b, De et al. 2005, Grossman et al. 2007). However, the role and the molecular mechanisms of p66Shc in this mode of regulation remain to be elucidated. In this review, we focus on discussing a novel functional role of p66Shc adaptor protein involved in steroid-related carcinogenesis, leading to its metastasis. This member of Shc protein family may serve as a new target for preventing tumor progression and metastasis.

**Role of Shc proteins in steroid-regulated tumor progression and metastasis**

Tumor progression and metastasis are the features of cancer. Cell proliferation, migration, and adhesion to the target tissues are the critical steps that allow tumor cells to obtain the metastatic phenotype. The process of metastasis requires the interaction of malignant cells with at least three distinct microenvironments, including the primary organ, the circulation or lymphatic channels, and the target organ where a metastatic lesion will develop (Radinsky & Fidler 1992, Mundy 2006).
showed that p52 Shc is responsible for transducing prostate cancer cell proliferation (Lee et al. 1997, Cooper et al. 2003). Within these microenvironments, several factors are involved in the metastatic cascade (Gopalkrishnan et al. 2001). Tumor cells, after reaching to target organs and tissues, establish as successful foci in the conducive environments. Subsequently, tumor cells proliferate in the new, supportive microenvironment as micrometastasis where they induce angiogenesis for maintaining the growth of new lesion. Induction of angiogenesis is necessary for successful metastasis to meet the nutrient requirements when the tumor size becomes more than 2 mm in size (Ellis & Fidler 1996, Gopalkrishnan et al. 2001). Cell proliferation and migration depend on intracellular signals transmitted by the growth factors and adhesion proteins within the extracellular matrix (Pages et al. 1993). Both these processes employ many common intracellular signaling molecules, e.g., Rho family proteins and ERK cascades (Pages et al. 1993, Olson et al. 1995, Anand-Apte et al. 1997, Klemke et al. 1997). It is evident now that Shc isoforms, specifically p52 Shc and p66 Shc, play a crucial role in cell migration and adhesion, in addition to their roles in mediating cell proliferation induced by growth factor receptor signaling (Fig. 4; Klemke et al. 1994, Hüttenlocher et al. 1995). These processes involve the rearrangement of actin cytoskeleton, the formation of new integrin substratum contacts, cell contraction, and the release of pre-existing cell-matrix contacts (Lauffenburger & Horwitz 1996). We will thus discuss the role of p52 Shc and p66 Shc in these processes and emphasize on steroid regulation.

**Role of p52 Shc in steroid-regulated cell proliferation and migration**

In addition to the classical role in mediating tyrosine kinase-activated pathways, p52 Shc functions as a primary adaptor protein for mediating the mitogenic signals of steroids at the non-genomic level in human breast and prostate cancer cells (Stevenson et al. 1999, Lee et al. 2004a). In prostate cancer, the data clearly showed that p52 Shc is responsible for transducing androgen-activated ErbB-2 signaling, which leads to prostate cancer cell proliferation (Lee et al. 2004a). This androgen-stimulated cell proliferation requires Y317 phosphorylation since p52 Shc Y317F mutant effectively abolishes androgen stimulation, and that inhibition occurs at certain phases of the cell cycle (Lee et al. 2004a). This observation on the role of p52 Shc in androgen action on prostate cancer cells is in parallel to that of p52 Shc in estrogen action on breast cancer cells, correlating with ErbB-2 activity (Stevenson & Frackelton 1998). In addition, vitamin D treatment caused a significant decrease in LNCaP cell growth, which is closely associated with the reduction in ErbB-2 activity and its downstream signaling mediator p52 Shc via dephosphorylating Y317, thereby emphasizing the involvement of tyrosine phosphorylation of p52 Shc in human prostate cancer cell proliferation (Stewart et al. 2005). Furthermore, the p52 Shc Y317F mutant blocks estrogen-induced cell cycle progression at both the G0–G1 and G2–M junctions (Stevenson et al. 1999). Additionally, in the presence of steroids, ER α-transfected HeLa cells exhibit chemoresistance, where p52 Shc mediates the anti-apoptotic activity of steroids and thus prevents those cells from etoposide-induced apoptosis (Kousteni et al. 2001). Clearly, p52 Shc plays a critical role in mediating steroid action, at least in part, via tyrosine phosphorylation non-genomic signaling.

p52 Shc may also be involved in the adhesion process of cancer cells. In rapidly adhering prostate cancer cells, e.g., LNCaP C-81 and PC-3 cells, the phosphorylation level of p52 Shc protein at Y317 correlates with ErbB-2 activation, higher than in slow-adhering LNCaP C-33 cells (Lee et al. 2004a, Yuan et al. 2007). Yet, both the adhesion rate and the Y317 phosphorylation of p52 Shc as well as ErbB-2 tyrosine phosphorylation level in slow-adhering LNCaP C-33 cells can be up-regulated by steroids. Thus, by correlating tyrosine phosphorylation and adhesion, p52 Shc may play a crucial role in the adhesion of cancer cells during steroid hormone-induced metastasis. Furthermore, a direct involvement of p52 Shc in breast cancer metastasis in transgenic mice that express polyomavirus middle T antigen with a mutated Shc-binding site has been demonstrated (Webster et al. 1998). Polyomavirus middle T antigen couples with and activates signaling molecules, such as Src, Shc, and phosphatidylinositol 3'-kinase (PI3K) for its oncogenic capacity. Importantly, in transgenic mice, which have metastatic tumors, the mutated p52 Shc-binding site on middle T antigen had reverted to the wild type and regained its function, thus emphasizing the potential importance of the functional p52 Shc in the process of metastasis in vivo (Webster et al. 1998). In addition, it has been revealed that in integrin signaling, Shc recruitment to the actin-associated cytoskeleton is important (McGlade et al. 1992, Schlaepfer et al. 1998, Wary et al. 1998). p52 Shc potentiates integrin signaling, and integrin ligation results in the activation of non-receptor tyrosine kinases, such as Src, Fyn, and focal adhesion kinase (FAK), which phosphorylates p52 Shc, leading to Ras activation and entering into the cell cycle (McGlade et al. 1992, Mainiero et al. 1995, Wary et al. 1996, 1998). Besides, the SH3 domain of...
Fyn interacts with the proline-rich region in the CH1 domain of p52Shc (Thomas & Bradshaw 1997) and the amino-terminal domain of p52Shc is shown to mediate the association of this adaptor protein to an actin-rich cellular fraction (Thomas & Bradshaw 1997). Additionally, a mutation of the PTB domain (S154P-p52Shc) abolishes integrin-induced p52Shc tyrosine phosphorylation where the SH2 domain of p52Shc is dispensable (Collins et al. 1999). p52Shc phosphorylation by c-Src can be augmented when the PTB domain binds to phospholipids (Zhou et al. 1995, Sato et al. 1997). These observations explain how the PTB domain localizes p52Shc to the membrane where it becomes phosphorylated by cytoskeleton-associated tyrosine kinases, which finally results in cell migration. It should be noted that these non-receptor tyrosine kinases, e.g., Src, closely interact with steroid hormone signaling pathway (Migliaccio et al. 2000, Guo et al. 2006). The molecular mechanism by which steroids induce cell adhesion and/or migration via p52Shc requires further investigation.

**Role of p66Shc in steroid-regulated cell proliferation**

In tyrosine phosphorylation signal transduction pathway, p66Shc conventionally known as an adaptor protein. Interestingly, p66Shc expression closely correlates with the growth rate of prostate cancer cells. For example, p66Shc protein levels in the rapidly growing cells, e.g., PC-3 and DU145, are approximately 4- to 13-fold higher than that in the slow-growing LNCaP C-33 cells and are over tenfold higher than that in even slower MDA PCa2b cells (Veeramani et al. 2005b). Recent data indicate that

![Image: Involvement of p66Shc and p52Shc in tumor metastasis. Growing primary tumors attract new blood vessels, i.e., angiogenesis, and lymphatic vessels, i.e., lymphangiogenesis, to promote local tumor growth, involvement of regional lymph nodes, and finally distant metastasis. In the process of metastasis after degrading or remodeling the basement membrane, metastatic cells detach from the primary tumor mass, intravasate, survive the stress of vascular transportation, and then evade host defense mechanism, which are in part regulated by the adaptor proteins p52Shc and p66Shc. Furthermore, targeting via microvessels and cell adhesion molecules, the increased phosphorylation of p52Shc and p66Shc proteins mediate the proliferation of tumor cells that metastasize to their preferred sites after extravasation into the target organ parenchyma, and are permitted to reside in the target tissue in which cancer cells respond to transendothelial growth factors from that specific organ. (The figure is adapted from La Porta 2000).]
p66Shc may play a critical role in mediating steroid-stimulated cell proliferation. In the presence of steroid hormones (androgen and estrogen), p66Shc protein level as well as cell proliferation rate is increased in hormone-sensitive human prostate (LNCaP C-33 and MDA PCa2b), testicular (Tera-1 and Tera-2), and breast (MCF-7) cancer cells, higher than those cells cultured in the absence of steroids (Lee et al. 2004b). Thus, steroids increase p66Shc protein level and concurrently cell growth.

To elucidate directly the functional role of p66Shc protein in steroid-regulated cells, both cDNA and siRNA approaches were employed (Veeramani et al. 2005b). In both p66Shc cDNA, transiently transfected cell population and stable subclones of slow-growing LNCaP C-33 cells, elevated expression of p66Shc correlates with increased cell proliferation. On the contrary, a decreased cell growth rate is observed when p66Shc protein is knocked down by its siRNA in the rapidly growing LNCaP C-81 and PC-3 cells. The data clearly establish the causal relationship of p66Shc protein and cell growth. Furthermore, p66Shc mediates growth stimulation by androgens (Veeramani et al. 2008). The clinical relevance of these data is supported by the observations that in prostate cancer archival specimens, p66Shc protein level is significantly higher in prostate adenocarcinomatous cells than in adjacent benign glandular cells (Lee et al. 2004b). Similarly, the expression level of p66Shc is elevated in metastatic breast, ovarian, and thyroid tumors and may serve as a useful prognostic marker for stage-IIA colon cancer (Jackson et al. 2000, Abdollahi et al. 2003, Park et al. 2005, Grossman et al. 2007). Nevertheless, some studies showed that p66Shc protein is down-regulated in the primary tumors of breast cancers (Davol et al. 2003). Due to the potential importance of p66Shc in carcinogenesis, further studies are needed to clarify the correlation of p66Shc expression with breast cancer. In summary, cross-talks between tyrosine phosphorylation signaling and steroid hormones have been well established (Weigel 1996, Meng et al. 2000, Grossmann et al. 2001, Guo et al. 2006, Kraus et al. 2006, Migliaccio et al. 2006, Weigel & Moore 2007); it is thus reasonable to propose that p66Shc mediates steroid action in steroid-responsive epithelial cells. We therefore hypothesize that the elevated level of p66Shc protein in steroid-related cancer cells plays a critical role in up-regulating those cancer cell proliferation and thus contributes to the tumorigenicity of those cancers (Fig. 2). The role of p66Shc in this mode of regulation requires further investigation.

Role of p66Shc in metastasis

Several studies have shown the involvement of p66Shc in cellular invasion, motility, migration, and/or metastasis. Jackson et al. (2000) have shown that xenograft bone metastasis of breast cancer cell line, MDA-MB-231, expresses p66Shc and its metastatic variant F-11 cells have a threefold higher p66Shc expression level. Furthermore, increased expression of p66Shc in lymph node-positive breast cancers correlates with an increased number of positive lymph nodes (Jackson et al. 2000). As higher levels of p66Shc protein are observed in breast cancer specimens with higher metastatic potential, it suggests the possibility that p66Shc influences cell motility and invasion other than the MAPK pathway (Jackson et al. 2000). Furthermore, Northev et al. (2008) have demonstrated that decrease in the ShcA levels or the expression of a dominant-negative ShcA mutant blocked TGF-β-induced motility and the invasion of Neu/ErbB-2-expressing breast cancer cells, thus exploiting the crucial role of p66Shc in the migration and invasion of cancer cells. In addition, elevated expression of p66Shc by cDNA transfection in LNCaP C-33 cells is associated with increased motility and invasion (Yuan TC, Lin FF & Lin MF, unpublished data). In parallel, LNCaP C-81 and PC-3 prostate cancer cells express higher levels of p66Shc and exhibit higher metastatic potential than LNCaP C-33 cells in xenograft animals (Veeramani et al. 2005b, Sebeger J & Lin MF, unpublished observation). The observations on the increased expression of p66Shc, but not p52Shc or p46Shc, in cell lines with higher metastatic ability and in the node-positive primary breast cancers also imply that p66Shc functions in metastatic pathway.

Integrins play a vital role in cancer progression because of their ability to regulate various intracellular signaling molecules that are essential for cell motility, cell survival, and proliferation (Ruoslhahti & Reed 1994, Hynes et al. 1999). It has been suggested that αvβ3 integrin plays a critical role in the metastasis of cancer cells to bone marrow (Cooper et al. 2002). αvβ3 integrin is expressed in breast and lung cancer cells that were originally derived from the bone marrow aspirates. αvβ3 is also expressed in highly tumorigenic, bone foci-derived human PC-3 prostate cancer cells, but not in low tumorigenic, lymph node foci-derived LNCaP cells (Zheng et al. 1999). The results of recent studies suggest that activated αvβ3 integrin regulates tumor growth in vivo by influencing VEGF expression. The up-regulation of VEGF expression depends on αvβ3 clustering where it promotes the recruitment of p66Shc and subsequently the phosphorylation of...
β3-associated p66Shc. Phosphorylation of p66Shc is a critical step for α,β3-mediated potentiation of VEGF expression and tumor vascularization in vivo (De et al. 2005). These findings provide insights into the role of α,β3 and p66Shc interaction as a regulator of tumor metastasis and angiogenesis. Thus, down-regulation of p66Shc inhibits VEGF expression as well as the tumor growth and angiogenesis in vivo (De et al. 2005). Although the molecular mechanism of p66Shc involvement in metastasis requires further investigations, it is hypothesized that p66Shc is involved in an early step of invasion or during cell motility (Jackson et al. 2000) and plays a role in steroid-regulated metastatic process.

Molecular mechanisms of p66Shc-mediated steroid action

In determining the prostate origin of metastatic cancers, cellular prostatic acid phosphatase (cPAcP) has been used as a biomarker, due to its cell-specific expression (Sakai et al. 1992, Chu & Lin 1998). The results of several studies collectively indicate that cPAcP exhibit the growth inhibitory activity by functioning as a PTPase (Lin & Meng 1996, Lin et al. 2001, Veeramani et al. 2005a). In parallel, the expression level of cPAcP negatively correlates with prostatic carcinogenesis, i.e., the level of cPAcP decreases in prostate cancer cells, lower than that in the adjacent non-cancerous cells (Reif et al. 1973, Foti et al. 1977, Loo et al. 1981, Chu & Lin 1998, Lin et al. 2001). Interestingly, in prostate cancer cells, the level of p66Shc protein shows an inverse correlation with cPAcP expression and has a positive correlation with Erb2 as well as ERK/MAPK activation (Veeramani et al. 2005b). In cPAcP cDNA-transfected stable subclone cells, p66Shc protein level is decreased and ErbB-2 as well as ERK/MAPK activity is diminished, correlating with decreased cell proliferation (Veeramani et al. 2005b). Conversely, elevated p66Shc protein level as well as ErbB-2 and ERK/MAPK activation is observed in cPAcP-inhibited LNCaP C-33 cells by inhibitors (Veeramani et al. 2005b). Similarly, in breast cancer cells, elevated expression of p66Shc protein correlates with ErbB-2 and/or MAPK1/MAPK activation (Stevenson & Frackelton 1998, Lee et al. 2004b). In addition, the up-regulation of p66Shc is shown in human ovarian, oral, and lung cancer cells expressing increased levels of ErbB-2 (Xie & Hung 1996). While the molecular mechanism of this inverse relationship between cPAcP and p66Shc protein remains to investigated further, it should be noted that the inverse correlation of cPAcP with p66Shc as well as ERK/MAPK activation is clinically relevant (Loo et al. 1981, Pontes et al. 1981, Solin et al. 1990, Sakai et al. 1993, Gioeli et al. 1999, Price et al. 1999, Lin et al. 2001, Lee et al. 2004b). Despite the fact that cPAcP may serve as a good prognostic marker for metastatic prostate cancers (Sakai et al. 1993), because of decreased cPAcP level upon tumor progression as well as increased p66Shc protein level in PCa cells, we contemplate that the p66Shc/cPAcP ratio may serve as a competitive surrogate biomarker for predicting the prognosis of advanced prostate carcinoma.

Furthermore, p66Shc may mediate steroid-stimulated cell proliferation via a non-genomic signaling pathway. In the rapidly growing cells, including steroid-stimulated cells, increased oxidative stress by the generation of reactive oxygen species (ROS) might contribute to the elevated p66Shc protein level. p66Shc protein can function as a stress sensor and is involved in regulating the intracellular level of ROS (Trinei et al. 2002). Activated metabolic reactions in the rapidly growing cells lead to increased production of ROS (Klaunig & Kamendulis 2004), which may in turn increase p66Shc protein levels to mediate oxidative stress signals (Fig. 3). Evidently, upon H2O2 treatment, in MEF cells or DLD-1 colorectal cancer cells, the expression level of p66Shc protein is increased (Trinei et al. 2002, Pacini et al. 2004). Additionally, ROS-induced phosphorylation of p66Shc protein at Ser-36 residue further promotes the generation of ROS (Nemoto & Finkel 2002, Orsini et al. 2004). Notably, recent studies reveal that p66Shc protein exhibits endogenous oxidase activity and its amino terminus contains a redox center, which is involved in electron transfer from cytochrome c to molecular oxygen and produces H2O2. This H2O2 mediates the opening of transition pores resulting in increased mitochondrial permeability and thus an abnormal high level of H2O2 can lead to apoptosis (Giorgio et al. 2005). Nevertheless, physiological levels of H2O2 function as growth stimuli. In addition to the above findings, p66Shc may also increase H2O2 production through Rac1-SOS-specific pathway (Khanday et al. 2006), possibly leading to androgen-independent cell proliferation (Knight-Krajewski et al. 2004). ROS therefore mediates diverse biological functions, including cell proliferation, cell adhesion, migration, and apoptosis (Fig. 3).

In prostate cancer archival specimens, the ROS level is higher in cancerous cells than in non-cancerous cells, correlating with the proliferation index (Lim et al. 2005). The functional role of ROS as a positive regulator of cell growth, including prostate cancer cells, is apparent in part by inhibiting the PTPase.
activity, and thus the corresponding RPTK can be activated (Finkel & Holbrook 2000, Liu et al. 2002, Lou et al. 2008, Veeramani et al. 2008). Furthermore, steroid hormones, e.g., androgens and estrogens, and growth factors, such as EGF, can up-regulate ROS production in cells, as such; cell proliferation is promoted (Sundaresan et al. 1996, Liu et al. 2002). This is consistent with our findings that androgenic treatment of LNCaP C-33 cells promotes cell proliferation via decreasing cellular PAcP and increasing p66Shc protein level and ROS production as well as ErbB-2 tyrosine phosphorylation (Meng et al. 2000, Veeramani et al. 2008). Therefore, an inverse correlation of p66Shc and cPAcP in prostate cancer cell is observed. Additionally, it has been demonstrated that ROS may play a critical role in the initiation and/or early progression of prostate cancer; as such, antioxidants are used in clinical trials for this cancer prevention (Veeramani & Lin 2007). Collectively, the data indicate that p66Shc can mediate non-genomic steroid action on cell proliferation and carcinogenesis including metastasis, while the molecular mechanisms require further investigations.

Conclusion and perspective

To discover the novel therapeutic targets for the prevention of tumor progression and metastasis, identifying the functional molecules that are involved in enhancing or suppressing these processes is one of the major challenges. The metastatic process in carcinogenesis is regulated dynamically by numerous factors, including growth factors, hormones, and extracellular matrices. To establish a metastatic lesion, tumor cells must complete all the steps in metastatic processes. Several studies have already shown that Shc proteins, including p52Shc and p66Shc, have marked effects on cell proliferation, invasion, and migration. In this review, we discuss a novel role of p52Shc and p66Shc in mediating steroid action on tumor proliferation and metastasis. We further present a novel non-genomic mechanism by which steroid signaling via p52Shc and p66Shc induces cancer cell proliferation, survival, migration and ultimately metastasis (Fig. 5). Nevertheless, the role of other key players engaged with p52Shc and/or p66Shc signaling pathway has yet to be identified. Thus, the molecular mechanisms by which p52Shc and p66Shc mediate steroid hormone-induced carcinogenesis require further investigations. Understanding the role of Shc adaptor proteins in cancer biology including the determination of the upstream regulators and downstream effectors of Shc functional pathways may lead to the development of novel anti-tumor strategies for targeting against steroid-induced epithelial cancers.

Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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