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Survivin: a dual player in healthy and diseased skin

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Short title:

Survivin in normal and diseased skin

Abbreviation list:

KSC: keratinocyte stem cells; TA: transit amplifying; PM: post mitotic; UV: ultraviolet; IAP: inhibitor of apoptosis; BIR: baculavirus IAP repeat.

Abstract

Survivin belongs to the inhibitor of apoptosis (IAP) protein family, and, in addition to the antiapoptotic functions, it also regulates the cell cycle. The survivin gene generates five major isoforms with diverse and opposite functions. Survivin is highly expressed in cancer and in few normal adult tissues, including skin. It is mostly detected in the nucleus of keratinocyte stem cells (KSCs), but it is also expressed in melanocytes and fibroblasts. Survivin isoforms are differentially detected in subpopulations of human keratinocytes, exerting contrasting activities. Survivin has an important role in the regulation of cell cycle in keratinocytes, and it protects these cells from anoikis and UV-induced apoptosis. In melanoma, survivin is abundantly expressed, and its subcellular localization varies depending upon tumor thickness and invasiveness. Survivin overexpression has been shown in squamous cell carcinoma (SCC), and it is also involved in UVB-induced carcinogenesis. The presence of survivin both in the nucleus and in the cytoplasm throughout the epidermal layers of psoriatic lesions suggests the involvement of this protein in the keratinocyte alterations typical of this disease. Additional studies on the expression of survivin isoforms and their subcellular localization in relation to function will confirm the key role of survivin in the skin and will open the field to new therapeutic strategies for many cutaneous conditions.

Introduction

The skin is the first important protective barrier against any external insult. To maintain this function, epidermis is continuously renewed. The regeneration process is ensured by the presence of a stem cell reservoir located in the basal layer of epidermis. Keratinocyte stem cells (KSC) have been shown to have a highest capacity of self renewal, quiescence, long persistence in the tissue, and resistance to apoptosis (Marconi and Pincelli 2010). KSC generate transit amplifying (TA) cells, which in turn, after a few rounds of division, terminally differentiate into post mitotic cells (PM). The progressive differentiation process leads to the formation of a stratified epithelium composed by the basal layer, the spinous layer, granular layer and the stratum corneum. KSC presence and function are essential for epidermal homeostasis, which is characterized by a fine balance between proliferation and differentiation/apoptosis of

keratinocytes.

Apoptosis is an evolutionary conserved process of autodistruction, which is started by cells in response to different stimuli, including UV-radiations, toxins, hormones and cytokines. Apoptosis is activated to eliminate cells characterized by highly damaged DNA, while it is inhibited to preserve important cell functions. Either intracellular or extracellular factors control apoptosis, leading to the activation of the intrinsic and/or extrinsic apoptotic pathway, respectively. Among extracellular activator of apoptosis are growth factors, hormones and cytokines, which act by binding to death receptors located on the cell surface, such as Fas and TNF receptors. On the other hand, intracellular signals include stress-activated players which control mitochondrial activity. At the molecular level, proteolytic caspases are the main effectors of apoptosis and are involved in both extrinsic and intrinsic pathway. The extrinsic pathway is centered on the activity of caspase-8, while the intrinsic pathway is mediated by caspase-9. Both pathways activate the effector caspase-3 that in turn activates a caspase3-specific DNAse which degrades cellular DNA. Apoptotic machinery plays a major control both in normal conditions and in diseased tissues, being at the origin of several skin diseases, including cancer (Lippens et al, 2009). It should be pointed out that in the skin, apoptosis and differentiation are two separate mechanisms, in that they appear to be independently activated in response to different stimuli and involve distinct genes (Gandarillas,1999). Apoptotic process is finely regulated by numerous molecular players, with both anti- and pro-apoptotic functions. Among anti-apoptotic members, IAP (inhibitor of apoptosis) proteins play a critical role. This review will summarize the expression and function of the most important IAP family member, survivin, in the skin, both in homeostatic conditions and in disease.

The dual function of survivin

IAPs family comprises Bruce, ILP2, Livin, survivin, NAIP, c-IAP1, c-IAP2, XIAP which are structurally different and, beside their common anti-apoptotic activity, appear to perform diverse functions. IAP ability to inhibit apoptosis by binding to caspases and blocking their activity is

guaranteed by a special domain named BIR (baculovirus IAP repeat), which is present in their structure in at least one copy. Unlike other IAPs, survivin not only inhibits apoptosis but also regulates cell division (Altieri et al, 2010). This dual action, which distinguishes survivin from the other IAP family members, is ensured by its unique structure. Survivin has only one copy of a modified BIR domain, which is used for the homodimerization of the protein and for its interaction with other chromosome passenger proteins. Phosphorylation of threonine 48 (T48) in the BIR domain influences survivin dual ability to inhibit apoptosis and regulate cell division. In addition, mutation of T48 in the BIR domain alters survivin ability to bind borealin, a chromosome passenger protein (Barrett et al, 2011). Survivin structure also differs from other IAPs at the C-terminal domain that is substituted by a coiled coil alpha-helix domain, which in turn is responsible for survivin regulation of cell division. As far as inhibition of apoptosis, few works had initially shown a possible association of survivin with effector caspases in a cell-free system (Shin et al, 2001). However, it has been recently demonstrated that, under physiologic conditions, survivin is not able to inhibit apoptosis by directly binding to caspases (Srinivasula et al, 2008). Under apoptotic stimuli involving mitochondria, survivin forms a complex with XIAP, thus increasing XIAP stability and its inhibitory activity against caspases (Dohi et al, 2004). Moreover, the binding of survivin to mitochondrial Smac/DIABLO, a pro-apoptotic factor, leads to a delayed Smac/DIABLO release in the cytoplasm, which then results in prolonged cell survival (Ceballos-Cancino et al, 2007). Another partner that facilitates survivin-mediated inhibition of apoptosis is HBXIP (hepatitis B X-interacting protein). Survivin and HBXIP form a complex, which in turn binds to pro-caspase- 9, preventing the activation of mitochondrial apoptosis (Marusawa et al, 2003). Although IAPs are generally considered anti-apoptotic proteins, the gene encoding for wild type survivin, named *birc5*, generates five major isoforms of the transcript by alternative splicing, survivin-2B, survivin-ΔEx3, survivin-3B, survivin-2α. Survivin-2α and survivin-2B mediate apoptosis, while WT-survivin, survivin-ΔEx3 and survivin-3B appear to be cytoprotective (Mahotka et al, 1999; Badran et al, 2004, Caldas et al, 2005a). Among survivin isoforms, survivin-2α is an example of regulation of survivin, in that it physically binds to wild type survivin, thus limiting its anti-apoptotic activity (Caldas et al, 2005a-b). Some of the isoforms are also involved

in the control of cell division (Knauer et al, 2007).

Survivin is the only member of IAP family which is also able to interact with the mitotic apparatus, by binding to microtubules through its C-terminal coiled coil domain, thus ensuring for a correct cell division (Srinivasula and Ashwell, 2008). Moreover, by recognizing and complexing with phosphorylated histone H3, survivin associates with the chromosome passenger proteins Aurora B, INCENP and Borealin, and the neoformed complex is recruited to the mitotic centromers, to assist and ensure proper chromosomal segregation (Kelly et al, 2010). Additionally, survivin localizes at the centrosomes of dividing cells where it binds to Cdk1, whose activation allows the cells to enter mitosis (O'Connor et al, 2000). Survivin knocked-down cells fail to activate Cdk1, thus demonstrating its fundamental role in mitosis entry (Canovas et al, 2010).

It has been proposed that the dual role of survivin is associated to different compartimentalization of the protein in the cells. Nuclear, cytoplasmic and mitochondrial pools of survivin have been previously described (Fortugno et al, 2002; Dohi et al, 2004). Nuclear localization seems to be linked to survivin capacity to regulate cell division, while mitochondrial survivin is associated to inhibition of apoptosis (Fortugno et al, 2002; Colnaghi et al, 2006). Nuclear survivin facilitates cell cycle progression by inducing exit of the cells from G1 checkpoint arrest and subsequent entry into S-phase, but it is not protective against apoptosis (Temme et al, 2007; Connell et al, 2008a,b). Recently, it has been proposed that localization of survivin in the mitochondria, which corresponds to a greater stability of the protein, is associated to oncogenic transformation, possibly increasing protection from apoptosis in cancer cells (Dohi et al, 2004). Mitochondrial survivin confers resistance to apoptosis by inhibiting activation of effector caspases (Dohi et al, 2004). On the other hand, cytoplasmic survivin, after exiting from mitochondria, once apoptosis is initiated, looses the cytoprotective ability. It is proposed that cytoplasmic survivin is not able to protect the cells from apoptosis because of post-translational modifications (Dohi and Altieri, 2005). Mutations in survivin nuclear export signal (NER), which is responsible for its localization in the cyctoplasm, results in loss of protection against apoptosis (Colnaghi et al, 2006). An extra-cellular pool of survivin has been recently detected, retaining both anti-apoptotic and proliferative activities (Bokarewa et al, 2005; Mera et al, 2008; Khan et al, 2009). Survivin release in the extra-

cellular space is mediated by exosomes and is induced by stress stimuli (Khan et al, 2011). In addition, extracellular survivin seems to play an important role in the pathogenesis of rheumatoid arthritis (Ahn et al, 2010), suggesting that this pool should be also explored in skin diseases. While survivin has been predominantly investigated in cancer, little is known on its expression and function in normal adult tissues, such as skin.

Survivin in healthy skin

Over the last 15 years, survivin has been an exciting field of research mainly because of its highest expression in human cancers. Overall, survivin is poorly or not expressed in normal adult tissues, while its expression dramatically increases in tumor cells, pointing to survivin as a putative “tumor specific antigen”. In the skin, survivin expression varies depending on the species analyzed. In rats, survivin is expressed both in fetal skin and in adult epidermis (Iskandar and Al-Joudi, 2006), while adult mice do not express survivin (Grossman et al, 2001). In human skin, survivin function has remained unclear for a long time due to several works showing the absence of survivin in human adult epidermis (Grossman et al, 1999). However, more recent papers have shown that survivin is indeed expressed in normal human skin, and it is localized to the cytoplasm of few cells located in the basal layer of the epidermis (Marconi et al, 2007; Botchkareva et al, 2007). In order to identify the subpopulation of epidermal cells expressing survivin, we isolated a population enriched in KSC (Jones and Watt, 1993). Survivin expression was mostly confined to KSC in culture, with an almost exclusive nuclear localization (Marconi et al, 2007). This is in line with nuclear survivin as the main pool that controls cell division (Fortugno et al, 2002) and with recent findings showing survivin colocalization with stem cell markers in other systems (Li et al, 2010; Gheisari et al, 2009). The capacity of survivin to clearly distinguish KSC from TA keratinocytes is quite unique. Indeed, other markers such as β_1 integrin and p63 have been shown to be more expressed in KSC, although still being present in TA keratinocytes. On the contrary, survivin behaves as an almost exclusive marker for KSC, and its expression is almost absent in TA and PM cells (Marconi et al, 2007).

Survivin isoforms have been previously detected in different tissues and appear to perform different functions. We have shown that all the isoforms are expressed in human keratinocytes. In particular, wild type survivin, survivin-2B and survivin-ΔEx3 are markedly expressed in KSC, while survivin-3B and 2α are down-regulated in these cells and are mainly expressed in TA and PM cells. This suggests that survivin isoforms may have different roles in human epidermis; in particular, survivin-2B and -ΔEx3 could exert putative anti-apoptotic functions, while survivin-3B and -2α may act as pro-apoptotic molecules in this system (Marconi et al, 2007).

Survivin is more expressed in human hair follicles as compared to interfollicular epidermis. In these appendages, survivin is expressed in ki67-positive keratinocytes located in the hair matrix and outer root sheets, and it seems both to protect the cells and to regulate cell division (Botchkarewa et al, 2007). Whether survivin is expressed in the stem cell pool located in the outer root sheet of the hair follicle remains to be investigated.

As previously mentioned for other systems, survivin is involved both in protection from apoptosis and cell cycle regulation. Regarding the role of survivin in human keratinocyte cell cycle, while survivin overexpression does not induce changes in keratinocyte proliferative activity or differentiation capacity (Grossman et al, 2001), survivin downregulation impairs the ability of these cells to proliferate and form colonies in vitro. These data suggest that survivin is fundamental to sustain cell cycle progression in human keratinocyte. Moreover, survivin is markedly upregulated during UVB-induced keratinocyte cell cycle arrest, and its inhibition sensitizes these cells to low doses of UVB, resulting in apoptosis (Dallaglio et al, 2009).

KSC are protected from apoptotic stimuli, such as detachment from extracellular matrix (anoikis) (Tiberio et al, 2002) and UV radiations (Dallaglio et al, 2009). When KSC undergo anoikis, survivin-3B and -2α are upregulated, while wild type survivin, survivin-2B and survivin-ΔEx3 are down-regulated. This suggests a possible role of survivin isoforms in keratinocyte anoikis (Marconi et al, 2007). Moreover, survivin protects human keratinocytes against UVB-induced apoptosis (Grossman et al, 2001; Dallaglio et al, 2009). Although immediately after apoptotic doses of UVB, survivin is markedly upregulated in human keratinocytes (Aziz et al, 2004), its expression decreases 24-48 hours after irradiation (Dallaglio et al, 2009), as also shown upon

UVA-induced apoptosis (He et al, 2004). This suggests that elevated levels of survivin help to protect cells from cell death (Dallaglio et al, 2009). Moreover, anti-apoptotic stimuli such as tea polyphenols and resveratrol downregulate survivin expression during UVB-mediated damage (Aziz et al, 2005), suggesting that survivin expression decreases when apoptosis is inhibited by external factors. Altogether, these data provide evidence that survivin is important for both cell cycle progression and resistance against apoptosis of human keratinocytes. Overall, endogenous survivin has important roles in skin homeostasis, possibly by acting as a major player to maintain the stem cell reservoir of interfollicular epidermis.

In human melanocytes, survivin expression and functions are controversial. In some reports, melanocytes seem not to express survivin both in interfollicular epidermis (Thomas et al, 2007; Liu et al, 2006; Raj et al, 2008) and in the human hair follicle (Botchkarewa et al, 2007). By contrast, we have recently detected survivin in normal melanocytes in culture (Figure 1a, b), and its expression is downregulated by apoptotic doses of UVB radiations (Figure 1c). Ectopic expression of survivin in melanocytes protects these cells from apoptosis, without affecting their proliferation (Thomas et al, 2007). Interestingly, survivin may also influence melanocyte pigmentation, which is reduced in vitiligo patients (Fujita et al, 2009).

Although survivin in fibroblasts has been given little attention, normal primary fibroblasts and cell lines, both from humans and mice, express survivin and are used as a tool for validation of new survivin-based therapeutic approaches (Blanc-Brude et al, 2003).

Survivin is also detected in other cell compartments of the skin, such as endothelial cells. Survivin is expressed in capillaries of normal epithelium, while it is upregulated in endothelial cells of granulation tissue (O'Connor et al, 2000). Following injury of the skin, endothelial cells revascularize the damaged area by upregulating survivin expression (Byun et al, 2007). In addition, survivin sustains endothelial cell survival and proliferation (Dell'Eva et al, 2007), and protects these cells from apoptosis (O'Connor et al, 2000; Kirkiles-Smith et al, 2004). Consistently, survivin-ΔEx3 isoform is directly involved in angiogenesis (Caldas et al, 2007). Finally, a cell-permeable survivin/survivin isoform delivery system has been proposed as a possible strategy to improve revascularization of skin graft transplants (Shu et al, 2007). Given

the critical role of survivin in cancer, its role in angiogenesis is worth to be further elucidated, as targeting survivin in endothelial cells may be a potential strategy to inhibit tumor-associated vessels growth (O'Connor et al, 2000).

Survivin in diseased skin

Survivin is over-expressed in the majority of human cancers, as compared to healthy counterparts, including lung, colon, uterus, brain, ovary (Altieri, 2003). Indeed, survivin appears to be an important prognostic marker and/or a chemoresistance predictive factor in several human tumor types, in that its high expression levels in neoplastic tissues often correlate with poor prognosis of the patients (Su et al, 2010; Taubert et al, 2010; Xiaoyuan et al, 2010; Zaffaroni et al, 2005). In particular, the nuclear pool of survivin, rather than cytoplasmic survivin, can act as an independent prognostic factor for the clinical outcome of some human cancer and can correlate with tumor resistance to common therapeutic approaches. However, this effect seems to be tumor type-dependent, as nuclear survivin is reported to be an unfavorable prognostic factor only in some tumors (Moon et al, 2003), while it seems to indicate a favorable prognosis in other forms of cancers, such as breast and gastric carcinomas (Kennedy et al, 2003; Okada et al, 2001). This is in line with what happens in the regression of cutaneous T-cell lymphoma, a disease characterized by migration of mutant T-lymphocytes to the skin. In this type of cancer, nuclear survivin is predictive of systemic disease being associated with tumor progression (Goteri et al, 2007). Because the two main pools of survivin are apparently independent in terms of mechanism of action and differentially important for cancer development and recurrence, the subcellular distribution of survivin is of clinical relevance (O'Connor et al, 2000; Xia and Altieri, 2006). Consistently, the recently discovered mitochondrial pool of survivin seems to have an important role in cancer biology. Indeed, mitochondrial survivin protects cancer cells from apoptosis and promotes tumor formation (Dohi et al, 2004). Similarly, extracellular survivin has been shown to have an intriguing role in cancer maintenance. Tumor cells are able to release survivin in the extracellular space via exosomes (Khan et al, 2011). Here, it is promptly captured

by neighbor cancer cells, influencing and enhancing their proliferation, invasive capacity and resistance to therapy (Mera et al, 2008; Khan et al, 2009). Normal stromal cells do not absorb extracellular survivin, suggesting a tumor-specific mechanism of auto-control.

Cutaneous tumors include both non-melanoma and melanoma skin cancer. In both type of cancers, survivin is overall upregulated as compared to normal skin (Chiodino et al, 1999; LoMuzio et al, 2001; Bowen et al, 2004; Park et al, 2004; Bongiovanni et al, 2009).

Melanoma

Human melanoma is the form of skin cancer responsible for the highest number of deaths/year and has an innate capacity to metastasize and resist to therapy. As for other malignancies, survivin is overexpressed in human melanoma cells as compared to normal melanocytes (Grossman et al, 1999), and seems to be a promoting factor for both early step melanoma formation in vivo (Thomas et al, 2007) and the migratory capacity of melanoma cells (McKenzie et al, 2010). During melanocyte transformation, both p53 and RB directly bind to survivin gene promoter, resulting in survivin overexpression in melanoma cells (Raj et al, 2008). Consistently, survivin has been recently identified as a metastasis-associated gene for melanoma (Kabbarah et al, 2010). In this respect, survivin expression in sentinel lymph nodes of melanoma patients significantly correlates with a poor prognosis, unlike other anti-apoptotic genes, further indicating survivin as a good prognostic marker for melanoma invasiveness (Gradilone et al, 2003). Survivin expression is higher in thick melanomas as compared to thin primary tumors (Kabbarah et al, 2010). Nuclear survivin is reported to be predominant in primary melanoma, dermal and congenital nevi, while cytoplasmic survivin is mainly expressed in metastatic melanomas (Vetter et al, 2005), suggesting that different distribution of the protein in the cells may correlate with the aggressiveness of melanoma cells. According to some studies, survivin seems to be exclusively expressed in the nuclei of malignant melanoma (Ding et al, 2006). In others, both cytoplasmic and nuclear survivin are detected in melanoma, although only the nuclear pool is significantly correlated to poor outcome of the patients (Piras et al, 2007; Piras et al, 2008; Chen et al, 2009).

It should be noted though that all benign melanocytic lesions seem to be more homogeneous regarding survivin expression, and the protein is consistently detected in these lesions (Florell et al, 2005). Although survivin is released in the extracellular space, human melanoma patients do not have increased survivin serum levels as compared to control patients. On the contrary, these levels dramatically increase when the patients undergo chemotherapeutic treatment (Tas et al, 2004).

Because survivin promoter is considered as a tumor-specific element for melanoma, this raises the possibility of using survivin as a target for melanoma therapy (Eustace et al, 2010). Indeed, survivin downregulation sensitizes melanoma cells to apoptosis (Li et al, 2006; Chawla-Sarkar et al, 2004; Pennati et al, 2002; Liu et al, 2004) and survivin upregulation is responsible of melanoma resistance to therapy (Qiu et al, 2005). Consistently, low levels of survivin expression in human melanomas predict better patients outcome (Takeuchi et al, 2005). Both immunological and peptide-vaccines targeting survivin have been used as putative therapeutic approaches against melanoma. Regulatory T-cells against survivin have been found in the blood of patients with metastatic melanoma and not in healthy individuals (Vence et al, 2007), supporting the fact that anti-survivin vaccines may be a good approach against this disease (Reker et al, 2004; Otto et al, 2005). Although in mouse models targeting survivin induces apoptosis in melanoma cells (Yan et al, 2006; Grossman et al, 2001), in patients, preliminary results were not as promising. YM155, a small inhibitor of survivin, did not induce a positive response in patients with stage III and IV melanomas (Lewis et al, 2009). On the other hand, better results have been obtained with therapeutic approaches based on survivin-targeting immune-therapy. Indeed, T-cells against survivin can persist in melanoma patients after complete remission of the disease (Hadrup et al, 2006). A case report shows that when patients with stage IV melanomas were vaccinated with a survivin-derived epitope presented by dendritic cells, T-cell response against survivin was high and reached melanoma metastasis (Andersen et al, 2001; Otto et al, 2005). It is also reported that some melanoma therapies can modulate survivin expression, causing cell death (Pennati et al, 2003; You et al, 2004).

Non-melanoma skin cancers

Non-melanoma skin cancers include squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and other less frequent forms. Survivin is overexpressed in human and mouse SCC as compared to normal skin (Bowen et al, 2004; Bongiovanni et al, 2009), and correlates with tumor aggressiveness and lymph node metastasis (LoMuzio et al, 2001). This is also observed in head and neck SCC where survivin overexpression correlates with tumor progression and resistance to therapy. Survivin is expressed in benign lesions, such as actinic keratosis (AK), and in malignant tumors, both in situ and invasive SCC (Park et al, 2004), although in tumor lesions its expression is more pronounced. By contrast, human BCCs weakly express survivin (Park et al, 2004; Bowen et al, 2004; Chiodino et al, 1999), suggesting the presence of a tumor-specific expression pattern of survivin in the skin. In sebaceous neoplasms, such as adenomas and carcinomas, only nuclear survivin is expressed, and it increases in aggressive tumors (Calder et al, 2008). In line with these findings, nuclear survivin seems to be the predominant form, being expressed in virtually 100% of human SCC lesions, while cytoplasmic survivin is present in less than one third of the cases (Bongiovanni et al, 2009).

In cutaneous SCC carcinogenesis, survivin seems to play a crucial role. UVB, as one of the major source of mutations in the skin, favors the survival of p53-mutated clones, while it induces apoptosis of normal cells. The proliferative advantage of mutated cells allows the colonization of the surrounding space leading to amplification of p53-mutated cells. When survivin is overexpressed in keratinocytes, no changes in cell differentiation and proliferation is observed. On the other hand, survivin expression further suppresses apoptosis increasing the number of neo-formed p53-mutated clones following UVB irradiation (Zhang et al, 2005). Interestingly, expansion of p53-mutated clones is impaired by survivin overexpression in mouse keratinocytes. This is in line with the reduced susceptibility to form papillomas of K14-Survivin transgenic mice (Zhang et al, 2005, Allen et al, 2003). These data suggest that survivin protective function against apoptosis favors formation of mutated clones, yet inhibiting their expansion. However, once the tumor is formed, survivin is able to convert benign lesions to invasive SCC (Allen et al, 2003),

thus playing a key role in the progression of the disease.

In tumor cells, survivin is highly expressed, sustains their proliferation and protects them from apoptosis. Common cancer treatments induce apoptosis by downregulating survivin expression (Roy et al, 2009). In line with this finding, resveratrol, a grape derivative protective factor against tumor formation, reduces survivin upregulation following UVB-induced carcinogenesis (Aziz et al, 2005). It remains to be determined whether survivin also changes its intracellular localization during apoptosis in cancer cells. We have preliminary data showing that in cutaneous SCCs, survivin shifts from the nucleus to the cytoplasm after treatment with imiquimod (Figure 2a-e). Being survivin expression and function tightly correlated, it is of paramount importance to elucidate the subcellular localization of survivin before and after treatment.

Merkel cell carcinoma

Merkel cells are the mechanoreceptors of the skin and can give rise to Merkel cell carcinoma, a rare and highly aggressive malignant tumor, with high incidence of recurrence. Both nuclear and cytoplasmic survivin are expressed in all Merkel cell carcinoma lesions, higher expression correlating with tumor recurrence and metastasis (Tucci et al, 2006; Kim and McNiff, 2008). In the nucleus, survivin colocalizes with the mitotic spindle markers suggesting a key role in the control of carcinoma cell proliferation (Kim and McNiff, 2008). Cytoplasmic survivin expression is detected in areas of Merkel cell carcinomas characterized by large cell foci, predicting for better outcome. On the other hand, nuclear survivin is associated with poor prognosis of Merkel cell carcinoma, further confirming that studying survivin intracellular distribution may allow to predict tumor aggressiveness (Kim and McNiff 2008,).

Psoriasis

Psoriasis is an immuno-mediated skin condition characterized by hyperproliferation and reduced apoptosis of keratinocytes. Survivin is expressed in 70-80% of psoriatic lesion and it is overexpressed in lesional skin, as compared to normal and uninvolved epidermis (Bowen et al,

2004; Abdou and Hanout, 2008). In some studies, survivin is only expressed in the cytoplasm of psoriatic epidermis (Markham et al, 2006), while in others, both nuclear and cytoplasmic survivin is observed. Moreover, the cytoplasmic pool seems to be the most frequent in psoriatic epidermis (Simonetti et al, 2009). Perinuclear survivin in lesional psoriatic epidermis is also reported, but does not appear to correlate with either proliferative or apoptotic activity of the cells (Bowen et al, 2004). While in normal human skin survivin is located in some cells of the basal layer, in psoriasis survivin is also expressed in the upper layers. In particular, nuclear survivin is expressed in suprabasal layers of psoriatic epidermis, while cytoplasmic survivin is localized both in basal and upper layers (Markham et al, 2006, Simonetti et al, 2009). Because survivin protects against apoptosis and regulates cell cycle, it is conceivable that it plays an important role in the pathogenesis of psoriasis. Survivin upregulation has been observed in the epidermis of psoriatic patients treated with narrow band UVB combined with etanercept, a TNF inhibitor, suggesting a survivin-dependent increased risk of skin cancer formation in psoriatic patients undergoing this kind of combined therapy (Gambichler et al, 2010).

Conclusions and future directions

We can conclude that survivin plays a critical role in human keratinocytes, both in terms of protection from apoptosis and regulation of cell proliferation. In the skin, survivin appears to identify KSC (Marconi et al, 2007), indicating survivin as one of the major markers to characterize and isolate these cells. Furthermore, given survivin expression and functions, it is likely that survivin is involved in the mechanisms of hyperproliferative skin diseases, such as cancer and psoriasis. Subcellular localization of survivin is indicative of disease progression and response to therapy. Indeed, nuclear survivin is often associated with poor outcome in skin cancer patients and with neoplasm recurrence. Thus, defining cellular and subcellular expression in the diseased tissues is of paramount importance. Moreover, clinical data associated with survivin distribution in diseased versus normal tissues, before and after treatment, could help in clarifying the role of survivin in the evaluation of prognosis of patients and recurrence of the disease. Targeting

survivin in its different cellular and subcellular locations will also provide a strong therapeutic strategy (Moon et al, 2003). In addition, it would be very interesting to better explore the role of the newly discovered survivin extracellular pool in normal and diseased skin conditions (Figure 3). Survivin isoforms are very important in regulating survivin activity. Some of these proteins interact with wild type survivin, either enhancing or inhibiting survivin-mediated protection from apoptosis. To date, little is still known about survivin splicing variants role in the skin. We showed that all survivin isoforms are expressed in normal human keratinocytes, the anti-apoptotic variants being more expressed in the stem cell pool of epidermis, probably cooperating for protection against apoptosis. Moreover, survivin isoforms are modulated following apoptosis of human keratinocytes, suggesting a potential role in the maintenance of epidermal homeostasis (Marconi et al, 2007). It will be critical in the near future to better explore the expression and function of survivin isoforms in normal and diseased skin.

Different experimental therapeutic strategies targeting survivin have been developed in the last few years. More studies need now to be done at the skin level to develop new treatments for inflammatory and neoplastic skin diseases.

Conflict of Interest

The authors declare no conflict of interest.

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Figures

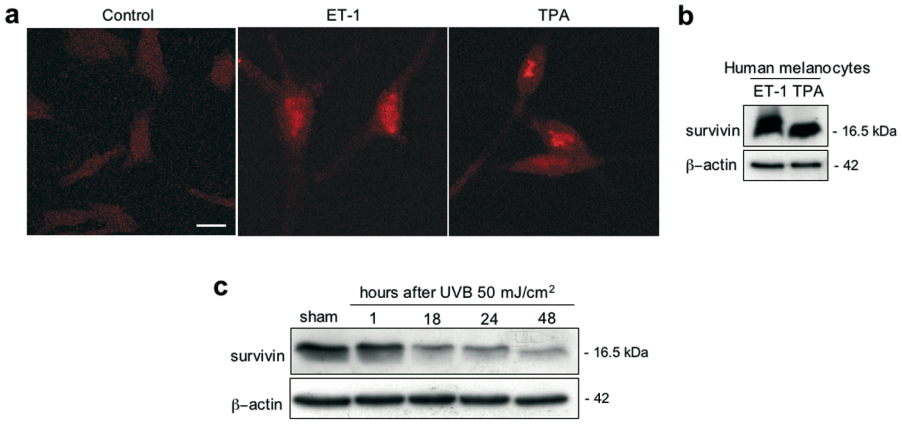
a
Control
ET-1
TPA
b
Human melanocytes
ET-1 TPA
survivin
- 16.5 kDa
β–actin
- 42
c
hours after UVB 50 mJ/cm2
sham
1
18
24
48
survivin
- 16.5 kDa
β–actin
- 42

Figure 1. Survivin expression in human melanocytes in culture before and after UVB radiations. (**a**) Normal human melanocytes were cultured with either endothelin or TPA. Cells were fixed in PFA 4%, immunostained at room temperature with anti-survivin antibody and analyzed by confocal microscopy. (**b**) Lysates from normal human melanocytes cultured with either endothelin or TPA, were analyzed by immunoblotting for survivin expression. β-actin was used as loading control. Survivin is expressed in human melanocytes cultured with either endothelin or TPA. (**c**) Human melanocytes were sham or UVB 50 mJ/cm^2 irradiated to induce cell apoptosis. At different time points after irradiation, protein extracts were separated on 18% polyacrylamide gel and immunoblotted with anti-survivin antibody. β-actin was used as internal control. UVB radiations induce downregulation of survivin expression in human melanocytes. TPA: 12-O-tetradecanoylphorbol-13-acetate; ET-1: endothelin-1. Bar= 25 mm.

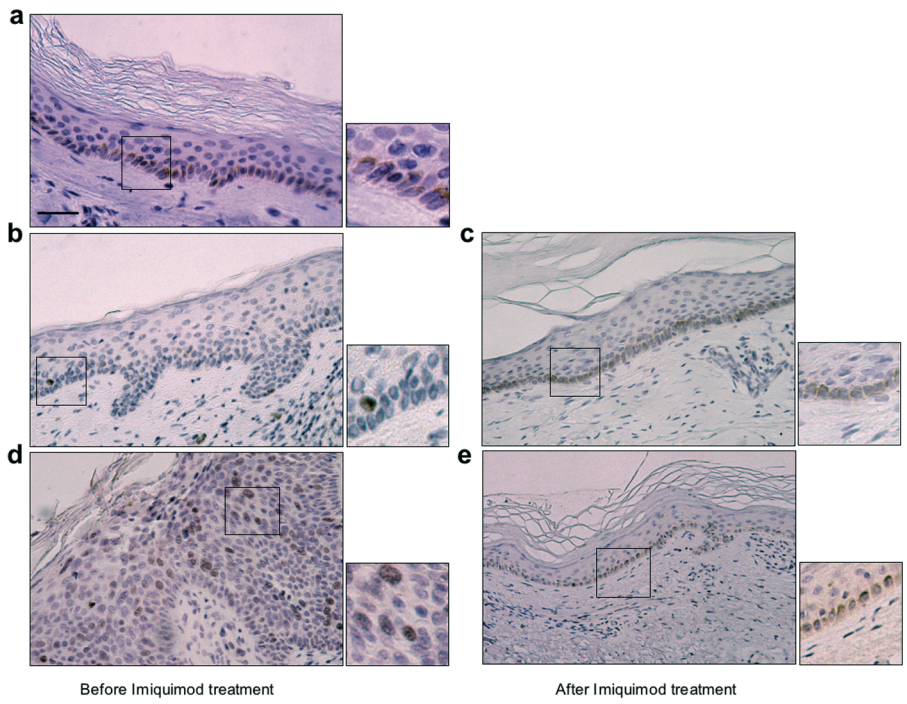

Figure 2. Survivin shifts from the cytoplasm to the nucleus of human cutaneous SCC cells after treatment with imiquimod. (a-e) Immunohistochemical staining for survivin of normal

human skin (**a**), Bowen disease (**b,c**) and cutaneous SCC (**d,f**), before (**b,d**) and after (**c,e**) treatment with imiquimod. (**a)** Cytoplasmic survivin is expressed in few basal keratinocytes of normal human skin; inset, magnified view of the area in panel. (**b,d**) Survivin is expressed in Bowen disease and cutaneous SCC in suprabasal keratinocytes, at the nuclear level; inset, magnified view of the area in panels (**b)** and (**d**). (**c,f**) Treatment with imiquimod, an anti-tumoral therapy for skin cancer, reverts the histological phenotype from suprabasal to basal keratinocytes and from nuclear to cytoplasmic localization, as seen in normal skin, after 8-week; inset, magnified view of the area in panels (**c)** and **(e)**. Bar=70 μm.

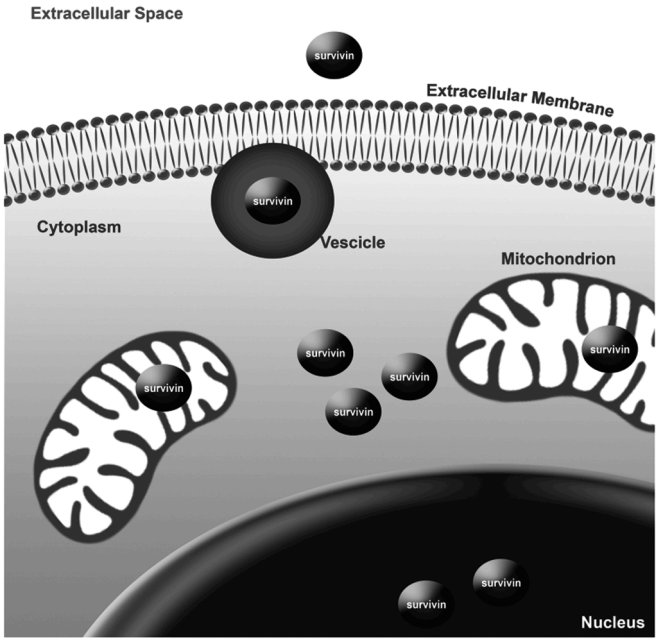

Figure 3. Survivin is localized in different compartments. Survivin is localized both inside and outside the cell. Inside the cells, three pools of survivin have been observed: the cytoplasmic, the nuclear and the mitochondrial pool. Recently, it has been shown that survivin is released in the extracellular space of cells by vesicles.