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Review Article

Modulation of T Lymphocytes by Tumor-Released Survivin

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Abstract

The tumor microenvironment is an area of intense interaction between normal and malignant cells. Factors and cell types within this environment can play a crucial role in the progression or regression of the tumor. Of primary interest are tumor-infiltrating T lymphocytes, which have been shown to have a key role in modifying the dynamics of the tumor microenvironment to promote or prevent tumor growth. While there is much *in vitro* and *in vivo* evidence for a modification of the tumor infiltrating T cell population toward a pro-tumor environment, what induces these changes within the tumor microenvironment has remained elusive. Our lab previously identified a role for the Inhibitor of Apoptosis protein Survivin as a secreted protein in the extracellular milieu, where it is capable of entering malignant cells and inducing a more aggressive phenotype. We hypothesized that tumor-secreted Survivin could be responsible for modulation of T lymphocytes in the tumor microenvironment. We first isolated tumor released Survivin and confirmed its ability to be taken up by T cells as it is by malignant cells by confocal microscopy, flow cytometry and Western blotting. Subsequently, we evaluated Survivin's affect on T cell proliferation and found that tumor-released Survivin impairs T cell expansion, but does not alter its activation after exposure to appropriate stimuli. Assessment of phenotypic changes within the cytotoxic and helper T lymphocyte populations showed an increase in anti-inflammatory type 2 T cells and a reduction in type 1 T cells, which correlates to what has been observed in cancer patients. Although often modified in the tumor microenvironment in patients, we did not observe any changes in regulatory T cells or Th17 cells in the presence of Survivin. The results of this study provide evidence of Survivin's role as an extracellular mediator of the tumor microenvironment, specifically its role in inducing a pro-tumor type 2 T cell population.

Keywords: Survivin, T Lymphocytes; Cancer; Exosomes; Apoptosis Inhibitor

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Introduction

Characteristics of Cancer Cells

Cancer is a complex disease involving the accelerated and uncontrolled proliferation of abnormal cells that invade normal tissues. While each type of cancer has different morphology, treatment and prognosis, several key features remain common throughout. Six hallmarks were originally described by Hanahan and Weinberg in 2000, most of them involving molecular changes within cancer cells themselves. The definition of these features and their consolidation into one picture of cancer provided a model through which cancer progresses, eventually gaining these functions to reach the malignant state [1].

The first of these hallmarks is the ability of cancer cells to replicate without limit, providing them with a never-ending proliferative capacity [1]. While normal human cells have a replicative capacity of approximately 60-70 divisions, malignant cells frequently exceed this limit, providing enough cells to form a tumor despite death of many of the pre-malignant cells along the way [2,3]. This function of cancer cells to continue to divide past the typical threshold of normal cells was found to be aided by induction of telomere-lengthening functions within these cells. In a normal cell, the chromosome ends, or telomeres, shorten every time the DNA is replicated, until eventually the ends are degraded to the point of inducing apoptosis of the cell [4]. By upregulating factors that preserve telomere length (telomerase, ALT) the normal proliferative capacity can be greatly exceeded by the malignant cell, resulting in tumor formation [1].

The next two hallmarks go hand-in-hand to push the unrestrained proliferation allowed by the telomere lengthening mentioned previously: self-sufficiency in growth signals, and insensitivity to anti-growth signals. In the normal cell, growth is only induced when a need of the body requires it. Some cell populations, like hematopoietic cells and gastric epithelial cells, replicate frequently due to wear and tear, while others replicate in the context of a specific need or after injury. The promotion of this renewal is mediated by growth factors and mitotic signals transmitted through surface and intracellular receptors [5].

On the opposing side, anti-growth signals in the extracellular matrix and milieu prevent the proliferation of cells when not needed as well as excessive growth of cells after the necessary divisions have occurred by inhibiting the cell cycle. Malignant cells alter both sides of this control mechanism to allow their unrestrained growth. By inducing their own mitotic signals by either production of growth factors (PDGF, TGF- α) or altering the receptor-signaling cascade (Her2/neu, Bcr-Abl) they can continue to proliferate even when exogenous growth factors have been withdrawn. Additionally, by disrupting normal pathways that block the cell cycle, such as the retinoblastoma (pRB) and p15^{INK4B} pathways, their endogenous growth factors push forward unchecked [6-8]. Throughout the process of DNA replication and cellular proliferation, errors frequently occur that must be detected by the cell to maintain cellular integrity [1]. Upon detection, cells are given the chance to repair the mistakes and continue their division or are forced to die if the errors cannot be corrected.

The final two hallmarks are vital to the advancement of malignancy past the point of a small focal lesion. To continue its growth the tumor must have access to nutrients and the ability to move past tissue barriers. The angiogenic potential allows for the tumor to maintain access to a nutrient blood supply by extending vessels and inducing new vessel growth into the tumor mass [1,2]. This is primarily mediated by increased cancer cell production of pro-angiogenic vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGF1/2) and reduced production of anti-angiogenic factors thrombospondin-1 and β -interferon [2]. With a nutrient supply available, the only remaining barrier to tumor growth is the boundaries of the tissue compartment it is contained within. Through production of matrix metalloproteinases (MMPs) and decreasing cell-cell adhesion molecules like E-cadherin, cancer cells can move away from the tumor and past the basement membrane barrier to invade nearby tissues as well as metastasize to distant locations [9,10]. This metastatic function is part of what makes treatment of cancer incredibly difficult, as simple surgical techniques cannot be used systemically to eradicate the malignancy.

The rapid and uncontrolled proliferation provided by the hallmarks we have discussed provide many opportunities for error that would likely lead to apoptosis in the normal cell. To prevent the likely death that would occur from replication infidelity in the malignant cell, mechanisms to inhibit apoptosis are necessary. It has been stated that evasion of apoptosis is the largest barrier a cancer cell must overcome, as studies have shown that induction of the previously mentioned hallmarks alone result in the apoptosis of a large proportion of the cells [2,11]. The evasion of apoptosis is mediated by multiple factors within the cell including the Inhibitor of Apoptosis (IAP) proteins and Bcl-2 family members [1]. By interfering with the caspase machinery and mitochondrial-mediated death, anti-apoptotic molecules can prevent death of malignant cells in response to replicative errors and cellular stressors.

As research in the field of cancer progressed, an updated version to this seminal review of cancer was released [12]. In 2011, Hanahan and Weinberg released "Hallmarks of Cancer: The Next Generation", bringing several new players to the forefront of the cancer research world. Several of these characteristics revolve around the interaction of the tumor and normal cells within the tumor microenvironment.

The tumor microenvironment

The role of fibroblasts: Within the body of the tumor there are a variety of cell types, both malignant and normal and as with normal tissue biology it is critical to understand the interactions between the variety of cellular players to understand how it functions. The presence of fibroblasts, endothelial cells and vessels are utilized by the tumor for protection, structure and nutrient support [12]. Fibroblasts play a vital role in normal tissues by production of the extracellular matrix and its ground substance and through the production of cytokines [13]. They are also crucial to proper wound healing, with fibroblasts and myofibroblasts initiating wound contracture and closure. In malignancy, fibroblasts help form the structural foundation for the tumor. Additionally, myofibroblasts, which normally make up only a small portion of normal tissue structure, are increased in tumors. Since myofibroblasts are also

increased in chronic inflammation-induced fibrosis, it is thought that inflammation in the tumor microenvironment increases the proportion of myofibroblasts [13,14]. The importance of this increase in myofibroblasts has been demonstrated in their ability to enhance tumor metastatic and angiogenic potential [15,16].

Immune cells: The tumor microenvironment is also home to a large number of immune cells, the role of which has been intently investigated. The role of immune cells in the progression or prevention of cancer and within the tumor microenvironment has been intently debated. Both anti-tumor and pro-tumor effects have been characterized and it now appears it is the balance of these factors and the tumor's ability to modulate this balance that determines what part the immune system will play in the development of cancer.

Macrophages: Macrophages are myeloid-derived immune cells that function to phagocytose and kill pathogens and present their antigens to the adaptive immune system. Within the tumor microenvironment macrophages are reprogrammed so that instead of activating the adaptive immune response, they quench it [14]. This can be explained by the dual nature of macrophages. As is seen in many immune cell populations, macrophages exist as either M1 or M2 cells. The type 1 or M1 phenotype is primarily pro-inflammatory and has an excellent capacity for killing cancer cells, presenting their antigens and activating an adaptive immune inflammatory response against the tumor [14]. These functions are mediated by the production of tumor necrosis factor alpha (TNF- α) and nitric oxide (NO). However, because these functions require the presence of oxygen, within the hypoxic tumor microenvironment, they are readily diminished. The type 2 or M2 phenotype of macrophages plays a more permissive role in the tumor microenvironment. Their production of growth factors such as VEGF promote tumor angiogenesis and sustenance [17]. Release of IL-10 and prostaglandins by M2 cells diminishes the immune response by Natural Killer (NK) cells and inflammatory T lymphocytes, providing a safe environment for the tumor [14,17].

Dendritic cells: Dendritic cells (DCs) are an important driving force in the immune response as

they are the most potent antigen presenting cells. The induction of an immune response or the production of anergy in adaptive immune cells is partly dependent on their maturity, with immature cells producing anergy and mature cells potentially activating the adaptive immune response [14]. Mature dendritic cells predominate in most tissues in order to provide activation of the adaptive immune system upon detection of a pathogen, however, within the tumor microenvironment there is a preponderance of immature DCs, while mature DCs are a rarity. Increased amounts of VEGF can impair DC maturation and it is suspected that other cytokines produced in the tumor microenvironment play an additional role [14].

B Lymphocytes: The role of B lymphocytes has been investigated in several different cancer types including breast, colorectal, melanoma and ovarian cancers [18-21]. It is currently unclear if the predominant function of B cells in the tumor microenvironment is pro- or anti-tumor [22]. Studies in breast cancer have shown that greater than 40% of patients have anti-tumor antibody production by B cells that correlate to a more favorable outcome [18,23]. Additionally, in lung and breast carcinoma, antibodies produced by B cells in the tumor microenvironment may aid in tumor elimination [23]. In contrast, studies in mice have shown that B cells may interfere with T cell eradication of tumor cells [14]. Determining the precise role of B cells in other malignancies is an area of intense research with many questions left to be answered, including if they primarily promote or hinder cancer progression.

T Lymphocytes: The role of T lymphocytes in malignancy is complicated at best. Multiple T cell phenotypes have been shown to play a role in the progression or elimination of cancer, depending on the cancer type [24-26]. Four main players have emerged in the T cell reaction to malignancy, and the balance of them can alter tumor progression. They include Type 1 and Type 2 T lymphocytes, regulatory T lymphocytes (Tregs) and IL-17 secreting T lymphocytes (Th17) [27,28].

Type 1 and Type 2 T Lymphocytes

Type 1 T cells are comprised of both CD4⁺ helper

and CD8⁺ cytotoxic populations, Th1 and Tc1, respectively. The defining characteristic of these cells is their cytokine profile (Table 1), which consists of tumor necrosis factor alpha (TNF- α), gamma interferon (IFN- γ), and interleukin 2 (IL-2) [29]. The type 1 T cell cytokine IFN- γ is vital to the anti-tumor response as it recruits and activates macrophages, as well as increasing the cytotoxic response by CD8⁺ T cells. Mice deficient in IFN- γ have been shown to spontaneously develop tumors and have poor tumor clearance, illustrating the importance of IFN- γ in anti-tumor immunity [30]. Production of IL-2 by type 1 cells increases the proliferation of nearby T cells, aiding the immune response by inducing clonal expansion of tumor-reactive cells.

The antagonists of type 1 cells are the type 2 Tc2 and Th2 cells, which release IL-4, IL-5, IL-6, IL-10 and IL-13 [27]. Type 2 cytokines prevent the differentiation of naïve T cells into type 1 cells and oppose the functions of type 1 cytokines. IL-10 decreases the cytotoxic response by decreasing antigen presentation by macrophages to Th1 cells, while IL-4 leads to decreased type 1 cell differentiation and IFN- γ production [31]. In cancer, IL-4 has been shown to prevent apoptosis of malignant cells, as well as increase the metastatic potential of breast cancer cells through polarization of infiltrating macrophages [17].

Peripheral blood and intratumoral T cells from cancer patients frequently have an imbalance in type 1 and type 2 T cell populations [25,32-35]. Type 1 responses are typically decreased with a concomitant increase in type 2 cell numbers and cytokine levels. The potential for metastasis and angiogenesis is increased by the presence of IL-4 and IL-6, respectively, both of which are produced by type 2 T cells [36]. This shift in the balance of type 1 and type 2 T cells has been correlated to poor disease progression, as the anti-tumor response is inhibited and a pro-tumor environment is created.

Regulatory T Cells

Characterized as CD4⁺CD25⁺CD127^{low}Foxp3⁺, regulatory T cells (Tregs) are important in preventing immune responses to autoantigens and over-activation of the inflammatory response [37]. Treg cells have been

Table 1: Cytokines released by type 1 and type 2 T cells and their functions in malignancy.

	Cytokine	Function
Type 1	Interferon- γ	Differentiation of naïve T cells into type 1 T cells Inhibition of the type 2 T cell response Increased cytotoxic CD8 ⁺ T cell response Activation of macrophages
	Interleukin-2	Induction of T cell proliferation Clonal expansion of anti-tumor T cells
Type 2	Interleukin-4	Inhibition of the type 1 T cell response Reduced interferon- γ production Prevention of cancer cell apoptosis Polarization of tumor-infiltrating macrophages Increased metastatic potential
	Interleukin-6	Increased angiogenic potential
	Interleukin-10	Differentiation of naïve T cells into type 2 T cells Decreased cytotoxic CD8 ⁺ T cell response

implicated in promoting tumor growth by suppressing activation of tumor-specific cytotoxic T cells due to their expression of auto-antigens by production of Transforming Growth Factor- β (TGF- β), cytotoxicity, and the induction of anergy [38]. In addition to suppressing the immune response, Tregs are also able to promote cancer stem cells. The regulation of cancer stem cells by Tregs is mediated by secretion of TGF- β , which promotes microsphere formation [39]. As with type 2 T cells, increased numbers of Tregs within the tumor correlates with poor prognosis and decreased responsiveness to immunotherapies [24,38,40-43].

Th17 Cells

Pro-inflammatory CD4⁺ Th17 cells produce IL-17 to recruit neutrophils and induce the production of pro-inflammatory factors in immune cells [44-46]. The role of Th17 cells in cancer is still unclear. The inflammatory Th17 response correlates with poor disease outcome in prostate, head and neck and colorectal cancer. This is hypothesized to be due to production of the anti-inflammatory mediator prostaglandin-E2 (PGE2) and pro-angiogenic hypoxia-inducible factor 1- α (HIF1 α) [39]. However, IL-17 knockout mice exhibited increased susceptibility to metastasis of colon cancer MC38 cells, demonstrating a possible anti-tumor function of Th17 cells [45]. Th17 cells reduce Treg cell numbers by lowering TGF- β release and increase

cytotoxic T cell function by secretion of IL-21 [39]. Although the precise mechanism of Th17 induction is still unclear, it appears that in malignancies initiated by inflammation, high numbers of Th17 correlate with poor prognosis due to their role in the induction of inflammation and angiogenesis [47]. In tumors that were not initiated by an inflammatory response, Th17 cells may be beneficial in activating a cytotoxic immune response to eradicate the tumor [47].

Although all of these changes that occur to the immune cells in the tumor microenvironment have been documented in patient and animal models, the precise mechanism that causes them is not well understood. While we have evidence for a role for hypoxia in skewing the tumor-infiltrating macrophage population, the same cannot be said for T cells. There are several hypotheses, one of which is the idea that one or more factors released by the tumor itself are likely a cause for this change in the T lymphocyte population.

Survivin

Survivin as a member of the inhibitor of apoptosis family

Survivin is a 142 amino acid protein and member of the Inhibitor of Apoptosis (IAP) protein family. As a member of this family it contains a baculoviral inhibitor of

apoptosis repeat (BIR) domain. It is so named because the original members of the IAP family (Cp-IAP and Op-IAP) were found to use this domain to bind and inhibit caspases in baculoviruses [48,49]. In humans, Survivin and its other IAP family members (XIAP, c-IAP1, c-IAP2, and NAIP) have been shown to inhibit programmed cell death [50,51]. However, unlike its other family members, Survivin does not contain the typical RING zinc finger or the caspase-recruitment domain (CARD) necessary for their anti-apoptotic function. Despite lacking these domains, Survivin is capable of preventing apoptosis signaled by TNF- α and Fas/FasL.

Anti-apoptotic function and role in cancer

Members of the IAP family typically prohibit programmed cell death through interaction with caspases, particularly activated caspase-3 and -7 [52,53]. NAIP, cIAP-1, cIAP-2 and XIAP have the ability to interact with caspases through their BIR domains while Survivin does not [54]. XIAP is the only IAP that has been shown to have the ability to bind and inhibit the effector caspases-3 and -7 and to a less degree, caspase-9 [55]. cIAP-1 and -2 can also bind caspases but without the ability to directly effect the inhibition of their enzymatic activities. However, their RING domains may, through a ubiquitin-dependent proteasomal degradation manner, allow for cIAP-1 and -2 to directly effect caspases by inducing their degradation [54]. Survivin's one BIR domain has not been shown to be able to bind caspases directly. With Survivin, the mechanism by which it inhibits apoptosis is not as clear. Some research has suggested that Survivin inhibits caspases-3 and -7 directly [56,57], while others have suggested that there is no interaction with caspase-3 [58]. Still others have suggested Survivin's anti-apoptotic effect is through inhibition of caspase-9 due to its ability to bind caspase-9 on the mitotic spindle and its ability to prevent staurosporine-induced death [59,60]. While the precise mechanism by which Survivin prevents cell death is uncertain, it is known that Survivin can prevent Bax- and Fas-induced apoptosis [50,57].

Survivin's overexpression has been documented in most, if not all, types of cancer, and high levels of it have been linked to poorer prognosis in small cell lung cancer, non-small cell lung cancer, hepatocellular carcinoma,

and colorectal carcinoma [61-64]. Survivin expression is also detectable in normal fetal development. In situ hybridization in chick embryos found that Survivin is expressed in the majority of fetal tissues, including lung, kidney and dorsal root ganglion. In mouse embryos, Survivin expression was ubiquitous throughout all tissues [65]. However, after fetal development Survivin expression is dramatically reduced or absent in mature human tissues. It is likely that through demethylation of the survivin gene promoter, malignant and pre-malignant cells are able to overexpress Survivin and evade apoptosis to go on to produce disease [66].

Survivin's function in cell cycle regulation

Survivin expression can be detected both in the cytoplasm and nucleus throughout the cell cycle, but its expression peaks during the G₂/M phase of the cell cycle [67]. This nuclear and cell-cycle regulated expression led to the idea that Survivin may play a role in cell proliferation. Indeed, upon further investigation it was found that Survivin is a component of the chromosomal passenger complex (CPC) along with Aurora-B and Borealin [68,69]. Its function in the cell cycle includes organization of the central spindle and correction of spindle attachment errors to allow cell division. This function is seen not only in malignant cells, but also in T lymphocytes. Upon T cell activation and clonal expansion, Survivin is upregulated to aid in the rapid proliferation that occurs [70].

Survivin in immune cell modulation

Survivin has a well-documented role in malignancy, but recent research shows it has an additional function in inflammation and autoimmunity. The amount of Survivin in the synovial fluid of rheumatoid arthritis patients has been correlated to a more severe disease progression [71]. It is thought that synoviocytes, which resemble fibroblasts, may be the source of Survivin production in patients with rheumatoid arthritis [72]. Additionally, when recombinant Survivin was added to immune cell cultures, it was found to upregulate adhesion molecules CD11b and CD11c on granulocytes and monocytes. Similar adhesion molecule profiles were observed in rheumatoid arthritis patients with high levels of synovial

fluid Survivin [73]. Though not as fully understood as cIAP-1, -2 and XIAP, research is beginning to produce a picture of Survivin's role in adaptive and innate immunity providing a niche that is still in need of investigation. However, it would appear that Survivin's role to date has been found more in adaptive immunity as it has been shown to have a vital role in T cell development, maturation and homeostasis [74,75]. Its role in T cell development and proliferation was accomplished using a conditional deletion of survivin allowing for T cell development to be investigated at every stage. When the survivin gene is deleted early in T cell development, the pre-T cell receptor proliferating checkpoint was affected and if it was deleted at a later stage both CD4⁺ and CD8⁺ T cells were reduced in number and immature [75].

Survivin as an exosomal protein

In addition to its cytosolic [76], nuclear [76] and mitochondrial [77] localization, Survivin has also been found in the extracellular milieu and in exosomes secreted by cancer cells [78,79]. Survivin has been shown to colocalize with Hsp70 in exosomes isolated from cancer cell-conditioned medium, demonstrating a novel pool of Survivin. Extracellular and exosomal Survivin has been shown to be taken up by malignant cells, resulting in altered function and increased aggressiveness [78]. Upon uptake into cancer cell lines, extracellular Survivin is able to induce proliferation and invasive potential as well as increase their resistance to UV radiation and chemotherapy. The exact mechanism that allows localization of Survivin into the exosome and its subsequent uptake into nearby malignant cells is not completely understood and is currently under investigation. However, it has been shown that increased cellular stress by sublethal doses of proton radiation enhances packaging of Survivin in exosomes, resulting in increased release of Survivin into the tumor microenvironment [79].

Survivin and the tumor microenvironment

Survivin has been shown to be an exosomal [79] and extracellular space [78] residing protein that when collected in exosomes and repleted on recipient cancer

cells *in vitro* can affect directly the phenotypes of these recipient cells [80,81]. Various cell types, such as B- and T-lymphocytes, dendritic cells, neurons, intestinal epithelial cells, as well as tumor cells, have been shown to release these small, 40-150 nm vesicles called exosomes [82-84] both *in vitro* and *in vivo*. Additionally, exosomes have been able to give additional indication of the function and biological roles of their cells of origin after identification of protein found both on their surfaces and within their lumens [82], making them ideal biomarkers for early detection, aggression and therapy efficacy.

Tumor-Released Survivin Alters the T Lymphocyte Population

We have demonstrated that the tumor-released protein Survivin can be isolated from conditioned medium, and upon addition to T cell cultures, is able to bind to and be taken up by T cells [74]. Like that described by Mera et al. [73] using recombinant protein, tumor-released Survivin is able to bind to the surface of T cells, and as demonstrated in our studies, is also taken up into T cells [74]. This data supports our hypothesis that Survivin uptake could occur within the tumor microenvironment as a means of modulating T lymphocyte function. The inhibition of T lymphocyte division by Survivin is in contrast to its effects on malignant cells [78]. While in malignant cells uptake of Survivin induced proliferation, T cell division is hindered. Based on the evaluation of activation markers CD25 and CD69, Survivin is not inhibiting T cell stimulation, but is possibly acting downstream of activation signaling. Additionally, no changes in activation-induced endogenous Survivin in the presence of FLAG-HA-Survivin were observed. Given the dramatic changes in IL-2 production in the presence of Survivin, it is possible that the mechanism of decreased proliferation is related to cytokine effects.

In addition to their decreased proliferative capacity, Survivin-exposed CD8⁺ T cells exhibited decreased cytotoxic function compared to controls. Many previous studies have demonstrated the inhibition of tumor-specific CTL function by T regulatory (Treg) cells [38,40]. However in our studies, Survivin did not significantly alter Treg numbers or function [74]. Because of these observations, we hypothesize that Survivin's inhibition

of cytotoxic function is independent of Treg-mediated CTL suppression. Based on our data, we believe changes to the cytokine profile and decreased IFN- γ production is likely responsible for this suppression. The release of Survivin may be one mechanism by which tumors evade T cell-mediated cytotoxicity.

Although Treg populations were not affected by extracellular Survivin, type 1 and type 2 T cell populations were significantly altered. The decrease in IFN- γ and IL-2 production by T cells, combined with the significant drop in IFN- γ^+ cells demonstrates an inhibition of the anti-tumor response. The importance of IFN- γ to immune surveillance has been shown previously in spontaneous malignancy in IFN- γ deficient mice. Additionally, it is frequently decreased in cancer patients [32,85-87]. The overall shift from a type 1 to a type 2 response was shown both by the decrease in IFN- γ^+ T cells and the increase in IL-4 $^+$, IL-5 $^+$ and IL-13 $^+$ T cells in response to tumor-released Survivin.

The importance of CD4 $^+$ IL-4 $^+$ cells in malignancy was recently demonstrated in a mouse mammary carcinoma model, in which production of IL-4 by helper T cells was able to polarize macrophages, resulting in increased lung metastasis [17]. Similarly, increased plasma levels of IL-13 in patients with pancreatic, gastric and esophageal cancers was positively correlated to increased numbers of myeloid derived suppressor cells [88]. Both these studies showed important downstream effects of the shift to a type 2 response, but did not examine the preliminary cause of the shift. Importantly, when combined with this study and our previous work showing Survivin-associated invasiveness [78], a more complete picture emerges with Survivin inducing the type 2 response, which can then lead to increased metastasis and complete polarization of the immune response.

Alteration of the immune response toward the type 2 phenotype has been demonstrated in many types of cancer, including lymphoma, breast, prostate and lung cancer, though the reason for this shift remains undetermined [25,32,33,85,89]. Similar studies have been performed looking at Th17 cells in cancer patients [28,45,47,90]. Unlike the Th1/Th2 shift, which is common across patients with a variety of cancer types,

the role of Th17 cells appear to be cancer-type specific. In prostate and ovarian cancers, increased numbers of Th17 cells in the tumor correlated with better patient outcome, demonstrating an anti-tumor role [28,90]. Conversely, in colorectal carcinoma, higher levels of serum IL-17 correlated to poor patient prognosis [91]. In our studies, no change in IL-17-expressing cells was observed after exposure to Survivin, unlike our observed type 1 and type 2 T cell shift.

Clinical Implications

Several studies have been concluded that emphasize that by inhibiting certain IAPs, inflammatory disorders may be controlled. In cIAP2 knockout mice, cytokine and chemokine release was halted due to increased death of inflammatory macrophages [92]. Furthermore, an IAP antagonist was able to suppress leukocyte infiltration into the injured liver of a concanavalin A-evoked hepatitis in mice by interfering with leukocyte-endothelial cell interactions through the inhibition of an important intercellular adhesion molecule ICAM-1 [93]. In another study, the XIAP inhibitor embelin suppressed bone erosion and inflammation in a mouse arthritis model through inhibition the NF- κ B activation [94]. Finally, a key molecular chaperone, Hsp90, with a role in pro-inflammatory vascular smooth muscle cells and atherosclerosis was shown to directly interact with Survivin. Therefore, a novel Hsp90 inhibitor mimetic, Shepherdin, was produced and shown to inhibit both Survivin and Akt function [95] leading to protection against vascular injury as well as vascular smooth muscle cell survival.

Alteration of the immune response toward the type 2 phenotype has been demonstrated in many types of cancer, including lymphoma, breast, prostate and lung cancer. Despite this important role in clinical malignancy the mechanism behind this shift has remained elusive. In these studies we showed that the polarization of the helper T cell response leading to a hindered pro-inflammatory response could be mediated by tumor-released Survivin. The overall shift from a type 1 to a type 2 response was shown both by the decrease in IFN- γ^+ T cells and the increase in IL-4 $^+$, IL-5 $^+$ and IL-13 $^+$ T cells in response to Survivin. These findings demonstrate a role for Survivin in mediating the shift

from a type 1 to a type 2 T cell response in the tumor microenvironment. The ability of Survivin to decrease the proliferation and cytotoxicity of T cells and alter the T cell response to malignant cells, as well as positively affect tumor growth and aggressiveness, makes it an important therapeutic target for the treatment of cancer.

Conclusion

Our studies isolated FLAG-HA tagged Survivin released from malignant cells and characterized its effects on normal T lymphocytes. Evaluation of Survivin's effects on T cells demonstrated its ability to diminish their proliferation without altering their ability to be activated by the proper stimuli. Additionally, Survivin diminishes cytotoxic T cell function by altering the cytokine environment. The large reduction in inflammatory T cells and cytokines, as well as the increase in type 2 T cells by Survivin coincides with what has been seen in cancer patients and supports our hypothesis that extracellular Survivin acts as an immune mediator. From our data [74] we can conclude that: 1) Extracellular Survivin can bind to and be taken up into normal T lymphocytes; 2) Uptake of Survivin diminishes clonal expansion of T lymphocytes; and 3) Survivin shifts the T cell population toward a type 2 phenotype, but does not alter the regulatory T or Th17 populations [74]. Further studies must be accomplished to determine the possibility of targeting Survivin to reestablish normal T cell differentiation and function. This strategy, as a means of reestablishing immune cell control of the tumor microenvironment, would allow the designing of mimetics as possible treatment options for patients with high levels of tumor exosome released Survivin.

Conflict of Interest

Both authors declare that they have no conflicts of interest to report.

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