Cancers **2014**, *6*, 1065-1097; doi:10.3390/cancers6021065

OPEN ACCESS

cancers

ISSN 2072-6694

www.mdpi.com/journal/cancers

Review

The Double-Edged Sword: Conserved Functions of Extracellular Hsp90 in Wound Healing and Cancer

Michael W. Hance, Krystal D. Nolan and Jennifer S. Isaacs *

Department of Cell and Molecular Pharmacology, Medical University of South Carolina, Hollings Cancer Center, Charleston, SC 29412, USA; E-Mails: hance@musc.edu (M.W.H.); dolek@musc.edu (K.D.N.)

* Author to whom correspondence should be addressed; E-Mail: isaacsj@musc.edu; Tel.: +1-843-792-8393.

Received: 13 March 2014; in revised form: 16 April 2014 / Accepted: 24 April 2014 / Published: 6 May 2014

Abstract: Heat shock proteins (Hsps) represent a diverse group of chaperones that play a vital role in the protection of cells against numerous environmental stresses. Although our understanding of chaperone biology has deepened over the last decade, the "atypical" extracellular functions of Hsps have remained somewhat enigmatic and comparatively understudied. The heat shock protein 90 (Hsp90) chaperone is a prototypic model for an Hsp family member exhibiting a duality of intracellular and extracellular functions. Intracellular Hsp90 is best known as a master regulator of protein folding. Cancers are particularly adept at exploiting this function of Hsp90, providing the impetus for the robust clinical development of small molecule Hsp90 inhibitors. However, in addition to its maintenance of protein homeostasis, Hsp90 has also been identified as an extracellular protein. Although early reports ascribed immunoregulatory functions to extracellular Hsp90 (eHsp90), recent studies have illuminated expanded functions for eHsp90 in wound healing and cancer. While the intended physiological role of eHsp90 remains enigmatic, its evolutionarily conserved functions in wound healing are easily co-opted during malignancy, a pathology sharing many properties of wounded tissue. This review will highlight the emerging functions of eHsp90 and shed light on its seemingly dichotomous roles as a benevolent facilitator of wound healing and as a sinister effector of tumor progression.

Keywords: extracellular Hsp90; wound healing; cancer; motility; invasion; EMT; MMPs; inflammation; LRP1

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1. Introduction

Cellular chaperones are essential for maintaining proteostasis, the balance between protein folding and degradation. Within this family of guardians, the abundantly expressed Hsp90 plays a critical protective role in countering protein misfolding and aggregation [1,2]. Hsp90 associates with a defined cohort of co-chaperones in an ATP-dependent manner to allow for precise regulation of its respective "client" proteins [3]. The advent of Hsp90 inhibitors has accelerated the discovery of hundreds of Hsp90 clients [4,5]. Although cytosolic protein kinases represent the predominant subclass of Hsp90 clients [6], many of which are implicated in malignancy [7–11], nuclear clients have also been characterized [12,13]. While the clinical utility of Hsp90 inhibitors has predominantly focused upon the targeting of malignant pathologies [3,14–16], the potential utility of these agents in protein folding pathologies such as neurodegenerative diseases has recently been reported [17]. Despite thousands of publications pertaining to cytosolic Hsp90 chaperone functions, it was only recently discovered that Hsp90 is also localized in mitochondria. This new locale for Hsp90 is predominantly observed in cancers, where it plays a critical role in regulating clients that oversee mitochondrial homeostasis and survival [18,19]. Taken together, a more comprehensive picture emerges, wherein Hsp90 orchestrates its protective effects and supports malignancy via regulation of numerous clients residing in multiple cellular compartments. Given the continuing evolution of Hsp90's diverse functions, it should come as no surprise that Hsp90 also possesses unique extracellular functions.

2. Out of the Box—Hsp90 on the Loose

2.1. Setting the Stage: Extracellular Chaperones

Nature provides us with continual surprises, including the discovery that a panoply of proteins demonstrate unexpected extracellular locations [20]. This phenomenon may enable cells to rapidly and efficiently respond to environmental cues and cellular stress. It is therefore a logical extension that several of the cytoprotective Hsp chaperones share this property of extracellular localization and function [21–23]. This geographic promiscuity endows chaperones with the ability to multi-task and broadens their sphere of biological function. To illustrate these trends, we will briefly highlight extracellular functions for the cytosolic chaperone Hsp70, its ER-resident paralog GRP78 (also known as BiP), and GRP94, the ER-resident paralog of Hsp90 (also known as gp96).

2.2. Extracellular Chaperones Exhibit Immunoregulatory Functions

The cytoprotective functions of chaperones are often subverted within the cancer context, a trend exemplified by GRP78 and GRP94, which support cancer cell survival, progression, and therapeutic resistance [24–26]. Similar pro-tumorigenic functions have been reported for cytosolic Hsp70 [27–29]. Interestingly, the extracellular counterparts of these chaperones exhibit intrinsic tumor-repressive and tumor-supportive functions. The pioneering studies of Srivastava and colleagues initially ascribed tumor-specific antigenicity to these surface-localized chaperones [30,31], a function associated with their ability to chaperone antigenic peptides and to activate anti-tumor innate immunity [32–38]. Surface Hsp70 has been shown to initiate antitumor T cell responses via cross presentation of its chaperoned peptides to MHC molecules [27,39–41].

Despite this tumor-alerting role, the surface localization of Hsps may not be entirely beneficial to the host. A variety of stresses commonly found in solid tumors, such as ER stress and hypoxia, may stimulate the extracellular localization of these conventionally intracellular chaperones [42–44]. Not surprisingly, their extracellular localization is preferentially associated with the malignant phenotype [45–53]. Moreover, beyond chaperoning tumor antigens, surface GRP78 functions as a multifunctional receptor to execute myriad signaling events impacting cellular proliferation and survival [21,24,52,54–57]. These surface-localized chaperones have also been shown to function as ligands in tandem with surface receptors to trigger signaling events and cytokine release [21,40,58,59] which may support inflammation-associated tumorigenesis [60]. Finally, both GRP78 and Hsp70 may be secreted from tumor cells, with functional roles in chemoprotection, inflammatory signaling, and cell invasiveness [21,24,38,53,57,61–64]. Thus, these surface and secreted proteins exhibit functional diversity, adopting roles as chaperones, receptors or signaling mediators to modulate host immunity and tumorigenic responses.

2.3. Immunomodulatory Functions for eHsp90

Extracellular Hsp90 also possesses tumor-repressive and tumor-supportive regulatory functions. The first sighting of eHsp90 was reported in 1986, wherein Hsp90α and Hsp90β were identified as tumor antigens in chemically induced mouse tumors [65]. This Hsp90 "antigen" conferred anti-tumor immunity to subsequent tumor challenge in immunized mice [31,65]. Mechanistically, surface eHsp90 may participate in the cross-presentation of antigenic peptides, with the capacity to regulate both innate and adaptive immunity [33,66–69]. Although the multifunctional LDL receptor-related protein 1 (LRP1), also known as CD91, was identified as the common receptor for the extracellular chaperones Hsp90, GRP94 and Hsp70 and [70,71], eHsp90-mediated cross presentation may also be regulated by its interaction with the scavenger receptor SREC1 [68], indicating its capacity to partner with additional molecules. Similar to the complexity observed with other extracellular chaperones, eHsp90 also exhibits functional diversity in adopting chaperone-dependent [64,72] and chaperone-independent functions [73–76]. As will become evident, eHsp90 partners with a growing list of receptors and adaptors to elicit pleiotropic signaling events. Taken together, these reports indicate that eHsp90 may have been designed as a powerful danger signal to elicit potent protective immune responses against infection and cellular stress. Although these early reports of tumor eHsp90 highlight its anti-tumorigenic immunogenic function, we, and others have demonstrated pro-tumorigenic functions for eHsp90, a topic that will form the basis for the remainder of this review.

3. eHsp90 as a Facilitator of Wound Healing

3.1. Stress Promotes Hsp90's Extracellular Location

Considerable controversy exists regarding the mechanism for Hsp90's transit to the extracellular space, all the more puzzling given the apparent lack of a signal peptide to direct its extracellular localization. Early reports documented the release of Hsp90 and additional chaperones following necrotic death [77]. Less profound breaches of membrane integrity were also shown to promote Hsp90 secretion [65], implicating a regulatory mechanism for release. Subsequently, mechanisms for

regulated secretion have been demonstrated, such as exosomal release [78–82]. A variety of stimuli are capable of inducing Hsp90's extracellular localization including DNA damage [79], oxidative stress [83], chemotherapeutic agents [84,85], growth factors and signaling mechanisms [80,86–88], heat stress [78] and hypoxia [82,89], as recently reviewed [90]. However, it is presently unclear whether these stimuli similarly invoke an exosomal mechanism for Hsp90 secretion. It is interesting to note that a majority of these stimuli are linked with cellular stress and likely to be present in a wounded environment, supporting the notion that eHsp90 functions in a protective capacity to buffer cellular stress. Therefore, eHsp90 has seemingly taken a page from the intracellular Hsp90 playbook by functioning as a guardian of extracellular homeostasis.

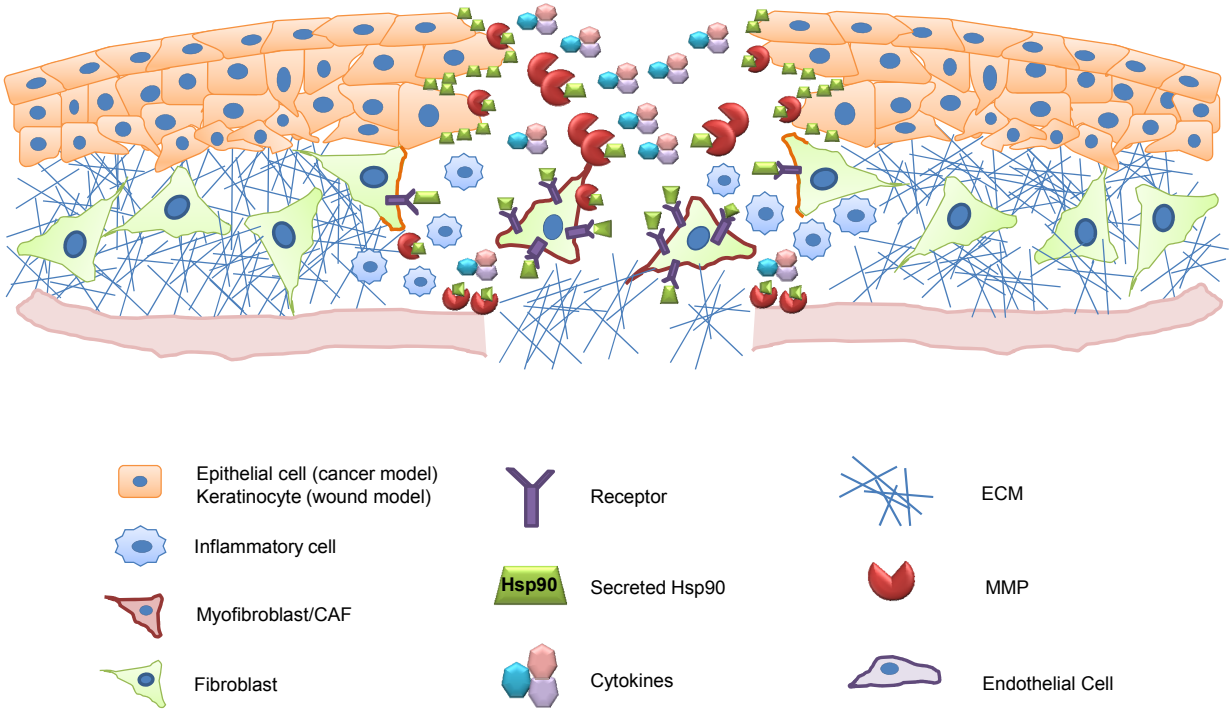
3.2. eHsp90 Is a Major Effector of Cell Motility

Although the intended physiological function of Hsp90 remains unclear, its adept response to cellular stress implies a conserved role in countering pathological conditions. Compatible with this notion, eHsp90 has been characterized as an essential mediator of tissue repair. Patsavoudi's group was the first to demonstrate this novel function for eHsp90 over two decades ago. Thomaidou *et al*. [91] utilized brain membrane fractions from developing rats to generate an antibody (4C5) against a cell surface antigen later revealed to be Hsp90α [92]. Intense immunoreactivity was also observed in Schwann cells following mechanical injury [93]. Importantly, these studies were the first to elucidate a role for eHsp90 in cell motility. Functional inhibition of surface Hsp90 via antibody blockade validated that eHsp90 played a major role in Schwann cell migration [92,94], significant given that Schwann cell motility is an integral component of tissue repair following peripheral nerve injury [95]. Work from this group indicated that eHsp90 also possesses an intrinsic developmental role, supported by the robust detection of surface Hsp90 in embryonic and early postnatal neuronal tissues with a high propensity for neuronal migration. Moreover, antibody-mediated blockade of eHsp90 prevented the motility of cells associated with developing cerebellar explants of the central nervous system [92,94,96]. Another report demonstrated that exogenously added Hsp90 protein stimulated neurite formation [97]. More recently, eHsp90 has been implicated in development of the cranial mesenchyme during neurulation [98]. Taken together, these findings suggest a physiological role for eHsp90 in morphogenesis and wound repair, processes with a shared reliance upon cell movement and tissue regeneration.

Dermal models of injury have revealed a conserved pro-motility role for eHsp90 in wound healing, as depicted (Figure 1). Key events associated with dermal injury include the mobilization of dermal fibroblasts and keratinocyte migration to promote re-epithelialization [99]. Li and Woodley demonstrated that eHsp90 was required for hypoxia-mediated migration of dermal fibroblasts [89] and keratinocytes [100] and for TGFα-mediated migration of keratinocytes [80]. This wound repair activity of eHsp90 was further validated by the ability of topically applied Hsp90 protein to accelerate murine skin wound closure and re-epithelialization *in vivo* [89]. It was further demonstrated that eHsp90's role in skin cell migration was dependent upon expression of the extracellular chaperone receptor LRP1 [80]. An eHsp90-LRP1 signaling axis similarly participates in hypoxia-dependent motility of skin cells [100]. The coupling of eHsp90 and LRP1 is an interesting partnership, as LRP1 is emerging as a key regulator of tissue damage and repair. LRP1 is upregulated during neural injury [101] and plays an important role in Schwann cell migration [102] as well as in inflammation and wound repair [103]. LRP1 has dozens of ligands [104], several of which are involved in pro-survival signaling

during injury [105]. We, and others have shown that hypoxia upregulates LRP1 expression [10,106–108], and cell surface localization [10]. Thus, cellular hypoxia is a stimulus capable of facilitating both LRP1 surface expression and Hsp90 secretion, events expected to cooperate and amplify the eHsp90-LRP1 signaling axis under pathological conditions.

Figure 1. Role of eHsp90 in wound healing. Schema depicts a dermal wound, with the upper layer representing epidermal keratinocytes. Wounded keratinocytes demarking the wound edges secrete Hsp90 (green rectangles). Surface-localized Hsp90 initiates signaling events that promote cell motility into the wound bed. Surface and secreted eHsp90 also activate MMPs to facilitate wound closure, cytokine release, and vascular repair. In tandem, eHsp90-dependent signaling and eHsp90-activated MMPs collaborate to activate fibroblasts to a myofibroblastic-like state (depicted in red). Myofibroblasts are prevalent in close proximity to the wound site, whereas their activation diminishes at more distal sites, concomitant with reduced concentrations of eHsp90. Myofibroblasts play a key role in synthesizing the provisional matrix and creating an inflammatory milieu that is also important for host defense. Key for cell types is shown below.



3.3. Evidence for eHsp90 in Matrix Remodeling

Although cell motility is important for wound repair, additional processes, such as wound contraction and matrix deposition, are essential components of the repair process. Modified fibroblasts, or myofibroblasts, at the injury site play a major role in these aspects of tissue repair [109]. A number of stimuli, such as TGFβ and mechanical stress, are well documented inducers of the myofibroblastic phenotype [110,111]. Myofibroblasts are characterized by expression of smooth muscle actin (SMA) and the appearance of SMA-containing stress fibers, cooperating events required for contractile force

generation and wound closure [112]. Myofibroblasts actively participate in connective tissue remodeling via their expression and deposition of extracellular matrix (ECM) proteins such as vimentin, fibronectin, and collagen for the provisional matrix. Matrix remodeling is also achieved via the concerted actions of proteolytic enzymes, such as matrix metalloproteinases (MMPs). Importantly, MMPs have been implicated in keratinocyte migration and wound contraction [113], and as will be further elaborated, eHsp90 is a major regulator of MMP expression and activity in diverse cell types [73,114,115].

Our recent demonstration that eHsp90 contributes to formation of myofibroblasts [76] lends further support to the notion that eHsp90 modulates matrix remodeling. Importantly, MMP activity was essential for several eHsp90-initiated myofibroblastic properties, and the function and/or activity of a subset of MMPs were under eHsp90's control. We demonstrated that eHsp90 regulates MMP-3 expression in eHsp90-initiated myofibroblastic cells [76]. Interestingly, MMP-3 is an important facilitator of the myofibroblastic phenotype [116], and eHsp90 was recently demonstrated to regulate MMP-3 activity during morphogenesis [117]. Thus, the ability of eHsp90 to promote a myofibroblastic phenotype adds mechanistic insights into eHsp90's complex roles in tissue repair. The ability of eHsp90 to orchestrate multiple components of the repair process, including myofibrobast generation, is undoubtedly a critical component of eHsp90's stimulation of *in vivo* epithelialization.

4. The Dark Side: eHsp90's Linkage with Malignancy

4.1. Sightings of eHsp90 in Cancer

Over the past decade, a more sinister side of eHsp90 has emerged, revealing a role for eHsp90 in malignancy. Whereas earlier studies characterized tumor eHsp90 as a protein capable of alerting the host immune system to danger [65], an avalanche of subsequent findings have supported the notion that eHsp90 promotes tumor progression. Further evidence of eHsp90's widespread role in tumorigenesis is provided by its frequent detection on the surface of diverse tumors including fibrosarcoma [114], breast [87], melanoma [118], ovarian [119] and neuroblastoma [120]. Studies from our group have demonstrated surface Hsp90 in tumor cells from both glioblastoma multiforme (GBM) and prostate cancer [10,121]. One pressing question is whether the *in vitro* identification of tumor surface Hsp90 has clinical relevance. In response to this valid query, Hsp90 has been detected on the surface of primary melanoma cells and resultant metastases [122]. Moreover, we have detected Hsp90 on the surface of primary prostate cancer specimens, and further identified the selective expression of transcripts associated with enhanced tumor aggressiveness in tumor cells with elevated levels of surface Hsp90 [121]. These latter studies provide compelling support for the notion that surface Hsp90 occurs within a clinical context.

Tumor cells also secrete Hsp90, indicating that both surface-localized and secreted Hsp90 populations contribute to malignancy. Although environmental stress stimulates Hsp90 secretion, Hsp90 is also preferentially secreted from cancer cells in the absence of exogenous stress, as shown in melanoma [123], breast [87], and our aforementioned studies in GBM and prostate cancers [10,121]. Clinical relevance for tumor-directed Hsp90 secretion is supported by demonstrations of elevated Hsp90 in patient serum, exemplified by the elevated Hsp90 levels in serum or plasma in patients with prostate, liver, breast, lung, pancreatic, and hepatocellular tumors relative to cancer-free controls [87,124,125]. Of particular interest, patients with metastatic disease exhibited the highest levels of serum Hsp90 [87,124]. The

presence of Hsp90 autoantibodies in patients with late stage ovarian cancer [119,126], breast cancer [127], and osteosarcoma [128] lends further support to tumor-derived circulating Hsp90. Although the functional relevance of secreted Hsp90 is not clear, a recent study demonstrated that a subset of colorectal cancer patients with elevated serum Hsp90 exhibited increased expression of integrin alphaV, a potential target of eHsp90, in the corresponding primary tumors, [129]. Further evidence for a functional role for secreted Hsp90 is demonstrated in that exosomal Hsp90 is responsible for a significant proportion of the pro-invasive activity of these secretory vesicles [81,130]. Finally, increased expression of rab27B, which regulates the exosomal release of Hsp90, is associated with breast cancer lymph node metastasis [130].

4.2. Role of eHsp90 in Cancer Cell Motility, Invasion and Metastasis

The widespread expression of eHsp90 in diverse cancers portends an important function. In keeping with this prediction, eHsp90 has emerged as a pivotal regulator of tumor cell motility, invasion and metastasis [131,132]. The first validation of eHsp90's role in cancer cell motility and invasion was demonstrated by functional blockade of eHsp90, wherein cancer cells treated with either anti-Hsp90 antibody or with derivatized cell impermeant small molecule Hsp90 inhibitors effectively suppressed tumor cell motility and invasion [92,114,118]. In recent years, a flurry of papers has reinforced the pro-motility and/or pro-invasive factor functions of eHsp90 in cancer [73,84,87,133,134] including our work in GBM and prostate cancer [10,121]. A number of studies have evaluated the effects of eHsp90 blockade *in vivo*. While one study demonstrated that this approach suppressed primary tumor growth [73], other studies reported no such effects on the primary tumor [87,118] However, all studies unanimously support the conclusion that blockade of eHsp90 function profoundly impairs tumor invasion and metastasis [10,73,87,118,135], findings that reinforce this fundamentally conserved role for eHsp90 in cancers.

4.3. eHsp90 Regulates MMP Activity

Tumor invasion is strongly implicated in tumor intravasation and extravasation, which represent important components of the metastatic cascade. Given that tumor metastasis causes the majority of cancer-related deaths [136], an understanding of how eHsp90 may regulate these processes has potentially high clinical relevance. Although several pathways are implicated in tumor invasion, activation of the MMP enzymes represents a critical proteolytic hub regulating cancer invasion and progression [137,138]. Interestingly, a number of reports identify eHsp90 as a major regulator of MMP activity. In fact, Hsp90α was detected in a regulatory complex with MMP-2 in one of the first reports illustrating the pro-invasive function of eHsp90 [114]. Subsequently, eHsp90α was found to regulate MMP-2 stability and/or activity in additional models [73,87]. eHsp90 has also been reported to regulate MMP-9 activity and tumor invasion as a component of an extracellular complex with the hyaluronan receptor CD44 [134]. Although the majority of reports implicate Hsp90α as an MMP-interacting pro-motility factor, other reports indicate that both isoforms may interact with MMP-2 and MMP-9 [115]. This interchangeability is not surprising given the high homology between the isoforms [139]. Some of this variability may also be explained by the various isoform-specific antibodies used to interrogate Hsp90 function, coupled with potential alterations in eHsp90's tertiary

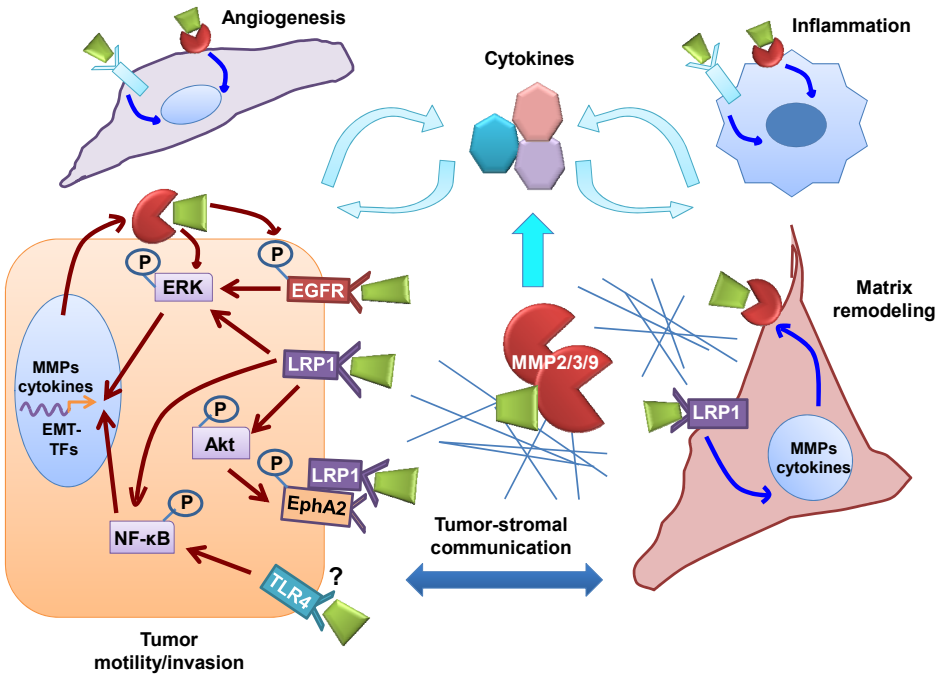
structure and/or interacting proteins that may sterically hinder antibody access. Recent reports indicate that eHsp90 may have a role in the activation of additional cell surface proteases involved in tumor cell motility [81]. Thus, although possible that Hsp90α and Hsp90β may possess distinct functions within some contexts, eHsp90's modulation of MMP activity is emerging as a powerful executor eHsp90's invasive functions.

4.4. eHsp90 Regulates Receptor Signaling

Extracellular Hsp90 cooperates with a growing number of transmembrane receptors to modulate signal transduction. Not surprisingly, eHsp90 partners with its immunomodulatory receptor LRP1 to drive cell motility in a number of cancers, as shown in colon cancer [129]. Our studies support conservation of an eHsp90-LRP1 pro-motility pathway in prostate and GBM [10,121]. In addition to LRP1, eHsp90 activates EGFR signaling in breast cancer, as shown by its interaction with the extracellular domain of HER2/Neu/ErbB2 [133], the ligandless co-receptor for EGFR family members [140]. This interaction was a requisite for ligand-mediated EGFR3/HER2 dimerization, signal transduction and invasive activity [133]. Another report demonstrated that eHsp90 facilitated EGFR endocytosis and stimulated receptor activity and cell migration in GBM [141]. Interestingly, this pathway required TLR4 activity, receptors that normally function as innate receptors critical for host defense. Our recent studies also support potential functional cooperativity between eHsp90 and TLR4 [142]. At least two studies have demonstrated that eHsp90 regulates integrin signaling, either by influencing its interaction with downstream intermediates [118] or by regulating integrin expression [129]. Finally, we demonstrated that eHsp90 was essential for regulating ligand-independent EphA2 activation and subsequent glioma invasion [10]. In addition to serving as a signaling conduit for eHsp90, several reports indicate that a subset of transmembrane receptors may serve a dual purpose in tethering eHsp90 to the cell surface. In support of this, cell surface CD44 was required for surface expression of Hsp90 [134]. Similarly, we observed that LRP1 was essential for the surface expression of LRP1 in GBM [10].

In addition to these direct mechanisms, a number of key intracellular signaling intermediates participate in eHsp90's pro-motility and/or invasive functions such as Src [118,141], PKCδ [141], NF-κB [129,141], and ERK [121]. The ability of signaling intermediates to form feed-forward circuits significantly increases pathway complexity. For example, eHsp90-mediated Src activation may potentiate receptor activation, as observed with integrins [118] and EGFR [141]. Compatible with this notion, we demonstrated that eHsp90-dependent Src activation in GBM facilitated AKT-mediated formation of a pro-invasive complex between EphA2 and LRP1 [10]. In addition, MMPs are well known effectors of cellular signaling [143] also capable of modulating eHsp90-directed signaling events, such as the liberation of growth factors to amplify eHsp90-activated receptor signaling [141]. Moreover, we have shown that MMP-2/9 is positioned both upstream and downstream of signaling intermediates such as ERK, indicating a complex bi-directional crosstalk mechanism [121]. Thus, a more comprehensive picture of eHsp90 signaling emerges, which includes eHsp90's direct interaction with cell surface receptors and proteolytic enzymes, signal transmission to downstream intermediates, and crosstalk between these intermediates and additional receptors and adaptor molecules, as depicted (Figure 2).

Figure 2. Role of eHsp90 in cancer. (Refer to cell type key in Figure 2). Summary of eHsp90 functions compiled from published reports in cancer epithelial and glioma cells (depicted by red arrows). eHsp90 interacts with a number of receptors (not directly confirmed for TLR4) to initiate signaling events converging upon AKT, ERK and NF-κB to facilitate cell motility. eHsp90-mediated activation of ERK and NF-κB promote epithelial to mesenchymal transition (EMT) activation in prostate and colon cancer, respectively. EMT activation collaborates with eHsp90-dependent signaling to further stimulate expression of MMPs and cytokines. Surface eHsp90 may also activate ERK through a variety of signaling intermediates. Surface eHsp90 signaling and MMP activation also elicits a CAF-like stromal reaction and promotes neoangiogenesis. CAF-like cells contribute to the inflammatory milieu, to the desmosplastic stroma, and to the angiogenic response. The earliest reports identified surface Hsp90 on inflammatory cells, thereby also implicating eHsp90 as a direct modulator of immune components in the cancer microenvironment. Finally, eHsp90 has been shown to interact directly with matrix components, indicating an accessory role in ECM remodeling. These collective events create an intricate web of cancer-stromal interactions that have the potential to create robust feed-forward networks that drive cancer progression.



5. Similarities between Wound Healing and Cancer

How is it possible to reconcile the seemingly disparate functions of eHsp90 as a beneficial facilitator of wound healing and as an effector of tumor progression? To understand this apparent dichotomy, it is important to appreciate the conserved pathways in wound healing and cancer. In 1858, Rudolph Virchow proposed his irritation theory for cancer. This astute observation was based upon that fact that neoplasms frequently developed at sites of chronic irritation. Subsequently, irritation or injury was recognized as a causative factor in cancer [144]. The pioneering studies of Mina Bissell validated this collaboration between injury and tumor progression [145]. The notion that chronic

wounds and associated inflammation are risk factors for tumor promotion [146,147] is well established, with inflammation now recognized as a major hallmark of cancers [148].

A singular goal of repair following tissue injury is to achieve wound closure and barrier restoration. The wound response is characterized by a complex series of integrated and overlapping components including cell motility, cell proliferation, matrix deposition and remodeling, inflammation and neoangiogenesis [99,149,150]. Cancers co-opt many effectors of the wound healing response, and features of a wound healing environment are commonly found in malignant and pre-malignant conditions [151–153]. Indeed, Dvorak is credited with the well known phrase that "cancers represent wounds that do not heal". As excellent reviews exist on these comparisons, a subset of shared processes will be briefly highlighted and subsequently discussed within the context of eHsp90 functions.

5.1. Hypoxia and Angiogenesis

Hypoxia in wounded skin is frequently due to vascular disruption and the higher oxygen demands of regenerating tissue. While transient hypoxia is required for vascular regeneration and repair in wounding [154], persistent hypoxia in the tumor microenvironment (TME) supports vascular permeability, tumor progression and metastasis [155–159]. Hypoxia also elicits a potent proinflammatory response [160–162].

5.2. Inflammation

Inflammation serves a number of essential roles during tissue injury. Inflammation activates the host immune system to elicit a protective response to guard injured tissue from pathogens [163]. Inflammation is also critical for stimulating neoangiogenesis and for enhancing the recruitment of stromal cells critical for the regenerative response [164]. Although designed to elicit a transient protective response to injury, the inflammatory milieu that persists in cancer supports tumor growth, angiogenesis, and tumor progression [148,165–167]. The recruitment of inflammatory cells also serves as an important source of proteases that contribute to degradation and remodeling of the extracellular matrix.

5.3. Matrix Remodeling

In normal wound healing, matrix deposition and remodeling are essential components of repair. Inflammatory cytokines released from the injured tissue promote formation of modified fibroblasts, or myofibroblasts, which play an essential role in many phases of the repair process [99,168,169]. The extracellular milieu of a wound is highly proteolytic, and myofibroblasts are major effectors of connective tissue remodeling due to their synthesis and proteolytic degradation of extracellular matrix (ECM) components [112,170,171]. In the cancer microenvironment, these myofibroblastic cell types are termed cancer-associated fibroblasts (CAFs). Similar to their counterparts in wound repair, CAFs are main producers of the tumor-surrounding ECM [172] and are key instigators of formation of the desmoplastic stroma that characterizes many advanced carcinomas. Whereas myofibroblasts resolve following wound repair [173], the constant remodeling of the tumor stroma sustains the myofibroblast population [172], which further supports the inflammatory and angiogenic millieu [174–177]. The pivotal role of CAFs in tumor growth, invasion, and metastasis is well documented [175,178–182].

5.4. Cell Motility and Invasion

Cell motility and invasion are critical components of the wound repair response [99,149]. In dermal wounds, epithelial cells, fibroblasts, keratinocytes, and other stromal cell types migrate inward towards the wound bed to achieve barrier restoration, wherein the provisional matrix serves as a scaffold for the migrating cells. In tandem, proteolytic enzymes such as MMPs diminish cellular adhesion to the basal lamina to facilitate the cellular motility required for repair [99,183]. These physiological repair processes are efficiently co-opted in cancer. In malignancy, the aberrant matrix serves as a highway for tumor cell motility, cancer cells acquire motile and invasive properties, and the constitutive ECM remodeling enables tumor escape [148,184,185].

In summary, many of the normally reparative processes associated with wound healing are constitutively activated in the tumor milieu [153,186]. The reactive stroma typically observed in cancers mirrors the myofibroblastic wound healing response [187–189] and an inflammatory stroma has been clinically associated with invasive disease and outcome [190]. Moreover, a gene expression signature indicative of wounding is predictive for cancer progression in a wide range of malignancies [191], a trend especially noted in breast cancer [190,191].

6. eHsp90 Is a Critical Factor in Wound Healing and Cancer

A picture emerges wherein eHsp90 orchestrates a continuum of events that support physiological wound healing and malignancy. This section will highlight the known molecular mechanisms for eHsp90 function and illustrate the duality of these processes in wound healing and cancer.

6.1. eHsp90 Regulates Angiogenesis

Hypoxia, a microenvironmental stimulus common to both wound healing and cancer, elicits Hsp90 secretion within both contexts [75,100]. Given that hypoxia in wound injury is designed to trigger vascular repair, it is not surprising that eHsp90, and in particular eHsp90α, affects a variety of cell types associated with vascular development. An early report indicated that eHsp90 modulated cellular signaling events in vascular smooth muscle cells (VSMCs) [83], while a subsequent report showed that eHsp90 promoted the motility of human microvascular endotheial cells (HMECs) and enhanced tubule formation [88]. Interestingly, this same group showed that a variety of growth factors, or the presence of ECM components, increased Hsp90 secretion from HMECs. This finding indicates that multiple stimuli coexisting within the wounded microenvironment stimulate reinforcing mechanisms to ensure Hsp90 secretion and subsequent repair of the damaged vasculature. To place this within a physiological context, Hsp90 was demonstrated to accumulate on the surface of regenerating tissue, and eHsp90 function was essential for neoangiogenesis in a mouse skin wound healing model [88]. Although the role of eHsp90 in the regulation of tumor vasculature is not well characterized, it was recently shown that eHsp90 regulates tumor angiogenesis *in vivo* [73]. Therefore, tumor secreted Hsp90 likely activates angiogenic pathways that are normally beneficial for vascular repair in wound healing. This notion is further supported by our prior studies demonstrating that in response to viral (pathogenic) challenge, eHsp90 exhibited a conserved ability to promote the secretion of angiogenic factors and sustain cell motility in dermal endothelial and cancer epithelial cells [192].

6.2. eHsp90 as a Pro-Inflammatory Factor

The earliest reports depicted eHsp90 as a protective mediator designed to alert the host to pathogenic stress [193]. Within this capacity, eHsp90 activates a number of inflammatory effectors and elicits the secretion of numerous inflammatory cytokines [66,77,194]. Recombinant Hsp90 induces the release of inflammatory cytokines in a wide range of epithelial and stromal cell types [76,77,192,194,195], indicating that cells have evolved conserved mechanisms to respond to eHsp90-mediated inflammatory pathways. By extension, eHsp90 utilizes similar conserved mechanisms to control inflammation in malignant tissues, with TLRs, NF-kB and LRP1 representing the key known pro-inflammatory eHsp90-regulated signaling nodes. TLRs play a major role in wound healing and cancer [196–198]. Of this family, eHsp90 appears to predominantly partner with the TLR4 receptor, as shown within the context of bacterial challenge [123,199]. NF-kB plays a central role in wound healing and cancer [147,163,196,200] and eHsp90 activates NF-kB in cancer cells [201]. We also reported that an eHsp90-NF-kB pathway is required for release of inflammatory cytokines in endothelial and prostate stromal cells [121,192], an activity consistent with its putative role in myofibroblast formation during the inflammatory phase of wound healing. The TLR4 and NF-kB pathways may collaborate in inflammation and cancer [202,203], and eHsp90 facilitates the secretion of inflammatory cytokines via a TLR4-NF-kB pathway in vascular cell types [195]. LRP1 is an acknowledged mediator of inflammation and wound repair, with additional roles in malignancy [103,204,205]. Although eHsp90-LRP1 regulated cytokines have not been well characterized, eHsp90 has been reported to signal through an LRP1-NF-kB pathway in cancer cells [201].

6.3. eHsp90 Regulates Matrix Remodeling

Matrix deposition and remodeling are essential components of wound repair. While this process subsides in wounding, the ECM is constantly remodeled in the tumor stroma, which resembles a chronically wounded reactive microenvironment. Fibronectin (FN) production is increased at healing wounds [206], and FN is also a major component of the cancer stroma [207,208], observations consistent with the notion that the tumor stroma shares many processes associated with wound healing. Recently, eHsp90 was shown to induce FN expression in breast and colon cancer cells [201,209]. Interestingly, eHsp90 associated with extracellular FN [209], indicating that eHsp90 may play a direct role in matrix assembly. ECM proteins including FN also stimulate Hsp90 secretion in endothelial cells [88], suggesting that a complex interplay between eHsp90 and the ECM is conserved in a number of cell types. ECM molecules play a protective role in buffering growth factors from degradation and providing biological latency [210]. Aberrant upregulation of proteolytic enzymes in the cancer stroma enhances the enzymatic degradation of basement membranes, which liberates bioactive molecules and correlates with metastatic potential [211]. MMPs, which are essential for wound healing, are also among the major regulators of this proteolytic turnover in malignancy [138,212].

Myofibroblasts and CAFs are vital to the ECM remodeling process in wound healing and cancer via their upregulation MMPs, including MMP-2, MMP-3 and MMP-9 [116,143,172,213,214]. Our findings indicate that eHsp90 may regulate MMP activity on several levels. First, eHsp90 initiates the formation of CAF-like cells [76], which are key culprits of the reactive stroma. Second, eHsp90 induces MMP-3 expression in CAF-like cells. Third, MMP activity is essential for manifestation of

several CAF-specific properties, indicating that eHsp90-dependent MMP upregulation is also a key event for generation and/or maintenance of CAFs. Moreover, we, and others have shown that eHsp90 regulates the expression and activity of MMP-2 and MMP-9 in tumor cells [73,87,114,115,121,134]. Thus, eHsp90 regulates proteolytic functions of tumor and stromal cell types, a toxic combination when considered within the context of the consequences for matrix remodeling, tumorigenic signaling, and tumor-stromal communication [184,215] (as depicted in Figure 2). In addition to generating the tumor reactive stroma, eHsp90-regulated MMP activity enables destruction of the normal interstitial architecture, which is a major facilitator of the aforementioned tumor invasion and metastasis.

6.4. eHsp90 Regulates Cell Motility in Injury-Induced and Cancer Models

The eHsp90-dependent regulation of cell motility and invasion is by far its most prominent role, highlighted by its crucial function in models of injury-induced migration and cancer. Functional conservation is strongly supported by the shared molecular events participating within these contexts. For example, the eHsp90-LRP1 signaling axis is essential for dermal cell motility following injury [80,100] and eliciting cancer cell motility and invasion [10,121,129]. Moreover, AKT activation was recently shown to be a key effector for eHsp90's migratory and *in vivo* healing functions [216]. By the same token, we identified an eHsp90-LRP1-AKT pathway as a primary regulator of GBM cell motility and invasion [10]. Our work further demonstrated that his eHsp90-LRP1-AKT pathway coupled to EphA2 receptor signaling, a pathway regulating the invasive activity of numerous cancers [217,218]. Although a direct eHsp90-EphA2 linkage has not yet been reported in wound repair, EphA2 and related family members are well known mediators of inflammation and regeneration [219,220]. LRP1 may also partner with the inflammatory mediator NF-kB to elicit eHsp90-mediated colon cancer cell motility [129], while NF-kB was shown to exert pro-motility effects via TLR4 in GBM [141]. These findings illustrate that a cohort of molecules with established inflammatory and/or or wound repair roles collaborate with eHsp90 to facilitate cell migration in a variety of cellular contexts.

EGFR and ERK are also emerging as major effectors of eHsp90-mediated cell motility in wounding and cancer. EGF is released by injury [221] and activation of the EGFR pathway is a central feature of wound healing [99,168]. TGFα, a ligand for EGFR, was required for Hsp90-dependent keratinocyte migration [80]. EGFR signaling was also important for eHsp90's pro-motility function in a number of cancer models [133,141]. ERK is essential for wound repair [222,223], and EGFR and ERK may participate in a signaling cascade [221,224]. We demonstrated that eHsp90-ERK signaling is required for the motility of both prostate stromal fibroblasts and prostate cancer cells [76,121]. In the latter instance, we showed that eHsp90-LRP1 dependent signaling was essential for ERK activation and for supporting eHsp90's motogenic effects. Finally, we demonstrated that MMP activity represents a critical component of eHsp90's ERK-dependent migratory activity. Collectively, these studies illustrate that a number of collaborative pathways reinforce and sustain the migratory functions of eHsp90 to modulate wound closure and cancer cell invasion.

7. The Big Picture: eHsp90 Initiated EMT Integrates Wound Healing and Cancer Progression

7.1. eHsp90 Initiates the EMT Program

Cells participating in wound healing and cancer activate shared pathways to transition from a sedentary to a motile phenotype. Activation of the developmental genetic program epithelial to mesenchymal transition (EMT) represents one of the primary mechanisms orchestrating this behavioral change. Many excellent reviews have discussed the broad similarities between the developmental EMT required for physiological morphogenesis and the pathological EMT associated with cancer [185,225,226]. Reactivation of the EMT program in malignancy is associated with increased tumor invasion and is considered a primary culprit for metastasis and cancer associated lethality. Receiving somewhat less attention is the comparison between EMT and tissue regeneration associated with injury. The wound closure process utilizes a mechanism analogous to EMT, characterized by the acquisition of mesenchymal morphology and increased migratory potential [227].

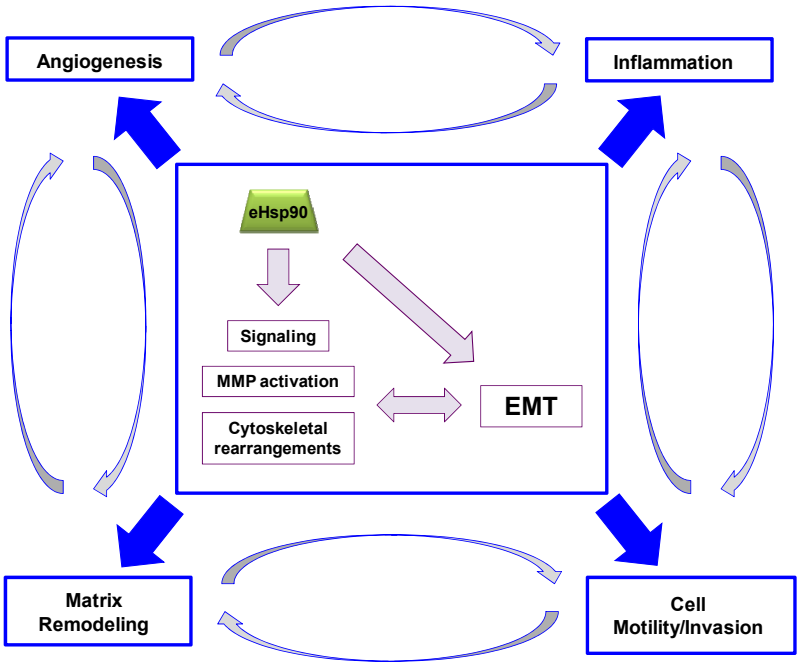
The full molecular reprogramming occurring during an EMT is primarily orchestrated by three major groups of transcription factors: the ZEB, Snail and Twist families. [228–230]. Reactivation of these EMT transcription factors (EMT-TFs) in cancer is a crucial step in initiation of the invasion-metastasis cascade. These EMT-TFs also play crucial roles in wound repair [227]. Remarkably, eHsp90 is emerging as a main orchestrator of the EMT program. We recently showed that eHsp90 is capable of inducing the transcription and expression of members from each of the 3 primary EMT-TF families in prostate cancer cells [121]. Shortly thereafter, eHsp90 was demonstrated to elicit an EMT response in colon cancer cells [201]. Thus, the ability of eHsp90 to direct EMT events considerably broadens our understanding and appreciation of its conserved functions in wound repair and cancer. We have herein highlighted eHsp90's central command of MMP activity and signal transduction, with consequent effects upon processes highly pertinent to wound repair and cancer, including angiogenesis, inflammation, matrix remodeling and cell motility, events inextricably linked with EMT activation in cancer and wound healing [148,227]. Within this context, we will revisit the known effectors of eHsp90 action within the broader context of the EMT program. A general schema reinforcing these trends is shown (Figure 3).

7.2. eHsp90's Pro-Motility Effectors are also Drivers of EMT

Increased cell motility is a well known hallmark of EMT, and a striking picture emerges with the realization that many of eHsp90's motogenic effectors are also facilitators of EMT, exemplified by TLR4 [231], EphA2 [232], EGFR [233–235], and MMPs [236,237]. Not surprisingly, these proteins exhibit dual roles in wound repair and tumor progression, a theme highlighting the conserved role of EMT in these processes. The relation between MMPs and EMT-TFs is complex. Although MMP activity may trigger EMT, EMT-TFs factors may also function as upstream regulators of MMPs, with their invasive function inextricably dependent upon MMP activity [238–240]. The ability of eHsp90 to activate MMPs also supports feed-forward mechanisms to drive EMT, as exemplified by the liberation of growth factors and activation of EGFR signaling [141,241] (and see Figures 2 and 3). Moreover, eHsp90 promotes cell motility in cooperation with the intracellular effectors NF-kB, AKT, and ERK [10,76,121,141,201,216]. These effectors serve as molecular hubs for a large number of

EMT-inducing signaling pathways [242] and may also drive EMT [243–246]. Our recent demonstration that ERK is essential for eHsp90-mediated EMT events [121] supports prior studies implicating ERK as an EMT activator [247–250], a function compatible with its known roles in development, wound healing and cancer [221,222,251].

Figure 3. Conserved functions of eHsp90 in wound repair and cancer. Central box depicts key molecular aspects of eHsp90 action highlighted in this review. These molecular events have the potential to elicit or support EMT activation, and EMT activation is reciprocally associated with heightened activation of these functions. Some of the key functional outputs of eHsp90 function are depicted in each corner of the box: Angiogenesis, Inflammation, Matrix Remodeling and Cell Motility and Invasion. As indicated, each of these functions may crosstalk and integrate with any of the other outputs to contribute to the highly complex tumor microenvironment.



8. Conclusions and Future Directions

The ability of eHsp90 to regulate inflammation, angiogenesis, matrix remodeling and cell motility is a perfect fit for its repair and regenerative properties. However, co-option of these activities in malignancy transforms eHsp90 into a powerful driver of tumor progression and metastasis. Tumor metastasis and wound healing share considerable phenotypic homology, including conservation of EMT activation. The ability of eHsp90 to regulate EMT events in wound healing and cancer offers considerable mechanistic insight into its orchestration of these processes. The secretion of Hsp90 from keratinocytes in wound healing or tumor cells in malignancy enables functional synergy between EMT-TFs and MMPs to facilitate remodeling and cell motility. While this transient response is useful for physiological wound closure and repair, these newly acquired properties endow cancer cells with the ability to invade and infiltrate the stroma, degrade the basement membrane, and ultimately access the vasculature to disseminate at distant sites. Interestingly, we reported that eHsp90 was highly

secreted in more aggressive and mesenchymal prostate cell types [121], a pattern shared with breast cancers [87]. Placed within this current framework, these trends may indicate that eHsp90 expression plays a critical role in enforcing mesenchymal behavior. This interpretation is generally supported by clinical findings wherein patients with metastatic disease exhibited increased serum Hsp90 expression [87,124]. It is noteworthy that stimuli regulating Hsp90 secretion, such as hypoxia, growth factors, and oxidative stress are also known inducers of EMT events [225,237,252]. This supports the notion that nature designed Hsp90 secretion as a reinforcing strategy for protection and repair, in part by eliciting EMT activation. The bi-directional relationship between eHsp90-mediated effectors and EMT-TFs creates a toxic reinforcing alliance that undoubtedly supports the tumor microenvironment, and hypoxia, inflammation, and MMPs are well known collaborators in the metastatic cascade [160]. Thus, it will be important to define factors regulating Hsp90 secretion upon exposure to microenvironmental stressors commonly found in malignancy.

Although targeting the broadly conserved pro-invasive functions of eHsp90 in malignancy is an appealing strategy, a more comprehensive understanding of eHsp90's role within the context of tumor immunity is warranted. The necrotic or injury induced secretion of Hsp90 is designed to alert the immune system, to initiate a survey of the tissue damage and to elicit repair mechanisms. However, the majority of studies implicating eHsp90 as a supportive factor in tumor progression have utilized immunocompromised mice. As such, no studies have yet evaluated eHsp90 immunoregulatory functions in physiologically relevant settings, a shortcoming that limits our understanding of the potentially redeeming tumor-suppressive properties of eHsp90. Inflammation has the paradoxical property of both enabling and suppressing tumor development [253,254]. The ability of eHsp90 to functionally and/or physically partner with the inflammatory mediators LRP1, TLR4 and NF-kB supports the premise that eHsp90 is simply executing its evolutionary conserved role as a master regulator of inflammatory responses. While eHsp90 may possess intrinsic tumor-suppressive functions, the tumor microenvironment undoubtedly hijacks eHsp90's pro-inflammatory functions, transforming eHsp90 into a major driver of tumor progression. Further studies are needed to understand the basis of this tipping point that converts eHsp90 into a malignant conspirator. Additionally, future work is required to define clear criteria for targeting the intracellular *vs*. the extracellular protein in cancers. Many chemotherapeutic drugs target signaling molecules and receptors essential for wound repair [227], further highlighting the shared molecular mechanisms in these processes. Now that eHsp90 is appreciated as a master regulator of tumor-supportive wound repair pathways, the rationale exists to explore clinically viable eHsp90-targeted approaches.

Acknowledgments

We thank Zihai Li and David Rowley for critical reading of this manuscript. Funding sources include R01 CA135297 and W81XWH-12-1-0324 (JSI), ACS 124154-PF-13-024-0-CSM (MWH), and NCI F31CA177015 (KDN).

Author Contributions

Jennifer S. Isaacs conceived the topic and general organization. Michael W. Hance, Krystal D. Nolan and Jennifer S. Isaacs wrote the manuscript. Krystal D. Nolan assisted with creation of figures.

Conflicts of Interest

The authors declare no conflict of interest.

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