β -thymosins in cancer: implications for the clinic

“ β -thymosin-based therapies have the potential to treat the two most prevalent causes of death in the USA – cancer and cardiovascular disease. Understanding the activities and mechanisms ... will be critical for their effective clinical use.”

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β -thymosins are a highly conserved family of approximately 40–44 amino acid peptides that regulate actin polymerization by binding and sequestering monomeric G-actin [1]. Actin filaments are essential for mitosis and cell motility, making their regulation an important component of cancer progression. β -thymosins are also active as extracellular mediators, where they are involved in a myriad of essential physiological responses to damage, including wound healing and hypoxic injury. During wound healing, high levels of β -thymosins are released by platelets into the wound milieu, where they promote angiogenesis, re-epithelization and moderate inflammation. In addition to regulating the cytoskeleton in cancer, the cancer microenvironment shares many features with a wound [2], making the increased β -thymosin expression observed in multiple cancers intriguing.

Currently, β -thymosins are in multiple clinical trials for treating pressure ulcers, venous stasis ulcers, epidermolysis bullosa, corneal wounds and acute myocardial infarction [3]. Two recent publications reviewing the clinical use of β -thymosins to promote wound healing and tissue repair raised the concern that β -thymosin therapy could have an untoward impact on undiagnosed cancer in these patients [1,3]. Indeed, patients who have been diagnosed with cancer are currently excluded from these trials. We propose that the limited risks may be outweighed by the benefits of treatment in cancer patients. Use of β -thymosins for wound healing will generally employ topical application. Furthermore, treatment for acute myocardial infarction, although systemic, would be limited in time, and therefore limited in risk of substantially promoting a cancer. However, until we better understand how extracellular β -thymosins act and whether they can promote cancer growth or metastasis, any long-term systemic treatments

may be of concern. Additionally, distinguishing between intracellular and extracellular actions of β -thymosins will be critical for development of any therapies, including anticancer therapies. Herein, we will summarize the evidence for β -thymosins facilitating the progression of cancer. Additionally, we will discuss the distinction between the extracellular and intracellular activities of these molecules and the potential role of each in cancer.

“Clinical use of β -thymosins to promote wound healing and tissue repair raised the concern that β -thymosin therapy could have an untoward impact on undiagnosed cancer in these patients.”

The three β -thymosins identified in humans are T β 4, T β 10 and T β 15. Increased T β 4 expression has been reported in breast, ovarian and uterine cancer [4,5], and is associated with metastasis in human colorectal, renal and lung cancers [6–8]. Experimentally, T β 4 expression enhanced tumor growth and metastasis in mouse fibrosarcoma and melanoma cells [9,10], and *in vivo* tumor growth and invasion of human colon carcinoma cells [11]. Conversely, antisense T β 4 expression reduced fibrosarcoma tumor formation and metastasis [9]. Together, this shows an importance of intracellular T β 4 overexpression in cancer.

Interestingly, T β 15 shows higher affinity for actin, relative to T β 4 [12,13], while exhibiting a more restricted tissue distribution. T β 15 is a prostate cancer biomarker in human tissues, and correlates with progression and adverse patient outcome [14,15]. Current screening for prostate cancer makes use of serum prostate-specific antigen (PSA) levels, which is confounded both by false-positives due to nonmalignant prostatic

conditions, and false-negatives from patients with PSA levels below the current cut-off. T β 15 is a diagnostic marker for prostate cancer when assayed in human urine, and markedly improves the specificity of prostate cancer diagnosis when combined with serum PSA. Preliminary data further suggest that urinary T β 15 may be a prognostic indicator of prostate cancer patient outcome [16], as seen for T β 15 in tissues. T β 15 overexpression has been reported in several other tumor types, including breast and lung cancer [17,18]. T β 15 RNA expression also correlates with progression, metastasis and disease recurrence in additional cancers, including cancer of the brain, as recently reviewed [19]. T β 15 overexpression increases transforming activity *in vitro* [20], while inhibition reduces tumor cell motility, tumor cell colony formation [14,20,21] and angiogenesis in the chorioallantoic membrane (CAM) assay [22].

Increased T β 10 expression has been observed in pancreatic, non-small-cell lung, renal, thyroid, colon, germ cell, breast, melanoma, uterine and ovarian cancers [5,6,23–26]. However, in contrast to T β 4 and T β 15, T β 10 appears to be a negative regulator of tumor development and progression. T β 10 peptide is antiangiogenic [27–29], while overexpression inhibits tumorigenesis and angiogenesis [30,31]. The contrasting roles of T β 10 as a tumor suppressor, and T β 4 as a tumor promoter, appear to be mediated via Ras activity, a critical modulator of tumor growth. T β 10 directly binds and inactivates Ras, while T β 4 does not [30]. Furthermore, opposing effects on tumor angiogenesis are likely mediated via T β 4 stimulation [10], and T β 10 inhibition of VEGF production [30]. The contradiction between increased expression in cancer tissues and the direct antitumorigenic effects of T β 10 might be best explained by a failed attempt at feedback inhibition. This surprising difference emphasizes the importance of distinguishing between correlative versus causal studies in understanding the role of factors in cancer progression. Additionally, this raises the exciting possibility of using therapeutic T β 10 in the treatment of cancer.

What are the potential consequences of extracellular β -thymosins in cancer? It is important to separate studies based on overexpression of β -thymosins in cell lines from those in which extracellular peptide is added, since current therapy is dependent on peptide treatment [30]. One might expect a tumor-promoting activity for T β 4 peptide, due to enhanced angiogenesis accompanied by increased HIF-1 α stability,

VEGF production and endothelial cell recruitment [10,32,33]. The addition of T β 4 peptide has previously been associated with increased expression of matrix metalloproteinase (MMP)-1, -2 and -9 in wounds and in different cell lines [34], and these proteins promote angiogenesis, as well as tumor invasion and metastasis.

“Would modulation of β -thymosins be a treatment strategy against cancer?”

The consequences of actin binding by intracellular β -thymosins have been best studied using T β 4. Regulation of the T β 4/G-actin reservoir via profilin competition was recently shown to facilitate T β 4 binding to integrin-linked kinase (ILK) in endothelial cell lamellipodia. This complex recruits and activates Akt2, resulting in MMP2 production [35]. In this model, the stimulation of ILK/PINCH/Akt by the addition of exogenous T β 4 was implied to have the same effects as T β 4 overexpression [36]. In contrast, systemic T β 4 administration into mice with infarcted hearts increased VEGF expression and PKC activation in the cardiac tissue 24 h after injection. Interestingly, in this study the authors speculate that PKC is responsible for Akt phosphorylation, independent of ILK [37]. Whether this would also apply to cancer cells is unknown. Akt activation appears to be a common mediator of both intracellular and extracellular T β 4 signals. Clearly, the extracellular activity of the β -thymosins leads to speculation regarding the existence of a β -thymosin receptor. Supporting evidence includes the rapid phosphorylation of c-Jun after addition of T β 4 peptide to the pancreatic cancer cell line Panc-1 [38]. However, to date, no cell-surface β -thymosin receptor has been identified.

Would modulation of β -thymosins be a treatment strategy against cancer? Altering intracellular β -thymosin expression or treatment with extracellular T β 10 peptides may both be feasible. While β -thymosins are overexpressed in many tumors, T β 15 shows the most restricted distribution in normal tissues, and may thus present a more specific target. A more difficult target may be T β 4 due to its vital role in all normal cells. However, future means of tumor-targeted therapies, coupled with knockdown strategies, for example, short interfering (si)RNAs, may circumvent this issue. In contrast to T β 4 or T β 15, the antiangiogenic activity of T β 10 peptide may lend itself to systemic treatment, targeting the extracellular tumor microenvironment.

β-thymosins clearly have great potential to modulate wound healing. In this context, should we be concerned regarding their biological role in cancer? Importantly, Tβ4 has been reported to have no role as a cancer initiator [1]. Essential to our understanding are experiments showing the effects of administering β-thymosin peptides to already established tumors. Such data have not been published. Although it would be fully expected to have an effect, to what extent this would promote tumor growth or progression would be important to understand. This information, together with better knowledge of the mechanism, may further enhance the value of β-thymosins in

the clinic.

β-thymosin-based therapies have the potential to treat the two most prevalent causes of death in the USA – cancer and cardiovascular disease. Understanding the activities and mechanisms of these small potent peptides will be critical for

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their effective clinical use.

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