Thymosin β as an Actin-binding Protein with a Variety of Functions

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According to current data, the thymosin β family is composed of 20 short (40–44 amino acid) peptides, but in a healthy human body only 2 are expressed – thymosin β4 and β10. Their most characteristic feature is the ability to form a complex with monomeric actin, thereby preventing polymerization into a filamentous form, hence the name Actin-Binding Protein (ABP). These peptides play numerous different functions. Among others, they affect the processes of carcinogenesis, differentiation and angiogenesis, influence metalloproteinase activity and accelerate wound healing. Moreover, significant biological activity has also been displayed by Tβ4 derived peptides: Ac-SDKP, the N-terminal fragment which is involved, inter alia, in stimulating angiogenesis and the inhibition of stem cell proliferation and Tβ4 sulfoxide, an oxidation product of one of the peptide methionine by hydrogen peroxide, which inhibit the development of inflammation. The properties of these peptides have potential applications in cardiovascular medicine, dermatology, ophthalmology and other medical areas (Adv Clin Exp Med 2016, 25, 6, 1331–1336).

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tension [12]. Long-term overexpression causes an increase in both G as F actin amount, which consequently does not change their mutual relationship. Moreover, in case of the long-term overexpression of Tβ4, an increase in the concentration of other cytoskeletal proteins such as myosin IIA, α-actinin, vinculin, and tropomyosin is also observed. At this time, the concentrations of total protein, DNA, profilin and actin depolymerizing agents are the same. It has been observed that cells overexpressing Tβ4 for a long time exhibit greater mobility and undergo stronger adhesion than control cells [13].

Tβ9, Tβ10 and Tβ15 have about two times higher affinity for G actin than Tβ4. On the basis of this fact, it is postulated that among the many mechanisms of increasing the mobility of the cells by reducing the concentration of the polymerized actin, there are at least two mechanisms related to thymosin: increasing concentrations of Tβ4 or replacement of Tβ4 with Tβ10 [11].

Thymosin β4 is present in the greatest concentration in the cells of all the β thymosins, that is, on average, about 0.4 mM (from 1 × 10⁻⁵ to 5.6 × 10⁻¹ M), which represents 70–80% of the total concentration of the thymosin β [14]. This peptide consists of 43 amino acids, has a mass of 4.9 kDa and its pI is 5.1. It is mainly located in the cytoplasm and is present in many tissues and cells, particularly in macrophages, blood cells except erythrocytes and tumor cells [14]. Tβ4 is also found in relatively high concentrations in the serous fluid of blisters, ulcers, wounds, blood serum and urine [15]. In adult human serum, it is present at a concentration of about 12.6 ng/mL (range 6.9–23 ng/mL) [16].

**Ac-SDKP**

Ac-SDKP is a tetrapeptide having the sequence N-acetyl-aspartyl-serine-lysine-proline. It is produced by the hydrolysis of Tβ4 at its N-terminus by the enzyme POP (propyl oligopeptidase) between the Pro4 and Asp5. It stops pluripotent stem cells in the G0/G1 phase, thus preventing their entry into the S phase. It is hydrolyzed by ACE (angiotensin-converting enzyme) [17]. In the event of it becoming blocked (e.g. by administration of captopril), Ac-SDKP concentration in the blood increases up to 5-fold [18]. It is credited with strong characteristics for inhibiting the expression of collagen and TGFβ (transforming growth factor β), limiting fibroblast proliferation, infiltration of macrophages, inhibiting the development of inflammation and accelerating neovascularization [18, 19].

**Tβ4 Sulfoxide**

In monocytes and neutrophils, in the presence of oxidizing agents such as H₂O₂, Tβ4 sulfoxide is generated, with oxidized methionine residue at position 6 [20]. In such form, thymosin has a lower affinity for monomeric actin [21], however, it is characterized by high biological activity. It is postulated that the compound modulates the immune cells, inhibits the activity of interferon-γ with a positive effect on wound healing, and other processes associated with inflammation [22].

**Functions of of Thymosin β4**

The main function of thymosin β is to maintain the pool of monomeric actin, which affects the dynamics of the cytoskeleton rearrangement, and therefore the mobility of cells, the capacity of differentiation, cell division, etc. Other functions are often associated with the fact that the protein is one of the ABPs, but not always. They are quite numerous and varied, and the main are included in Table 1. Some of the most important of them will be described in more detail below.

**Participation of Tβ4 in Angiogenesis**

It has been discovered that the thymosin family, despite the large structural homology, has peptides in its group with opposite properties relating to regulating angiogenesis. It was demonstrated that Tβ4, Tβ15, thymosin α1 and α prothymosin accelerated angiogenesis, but Tβ10, Tβ9 and parathymosin α, which differ from Tβ4 by 10 amino acids (outside actin-binding motif), significantly inhibited this process [38].

It is postulated that Tβ4 is of such importance in angiogenesis that if there is no active protein as a result of gene damage or disorder in gene expression, it induces disturbances in cell migration, differentiation, causes hemorrhaging and partial embryonic lethality. Rosseutsch et al. demonstrated this experimentally on germline null for Tβ4 and a second line in which Tβ4 was knocked down by endothelial-specific expression of Tβ4 shRNA [39]. In the work, arguments were shown that endothelial Tβ4 stimulates differentiation of mesodermal cells to a mature mural by enhancing the activity of TGFβ signaling. It was also demonstrated that retardation of the TGFβ pathway correlates with the penetrance of hemorrhage in Tβ4-null aortas and modification in downstream signaling. The authors concluded that this information may help analyze the reasons for congenital abnormalities of the blood vessels, which in adulthood could
manifest in a tendency to form aneurysms and lethal bleeding [39].

It was also found that Tβ4 not only plays an important role in the formation of blood vessels, but also may be important in the regeneration of damaged vessels. Shelton and Bader [40] reported that Tβ4 mobilizes mesothelial cells for tissue repair. An experiment demonstrating this phenomenon consisted of potentiating omental grafts with agarose beads soaked in Tβ4. This peptide stimulated omental cells to migrate and differentiate into smooth muscle cells in the damaged area, wherein the mesothelial cells of the graft were integrated into the wounded vessel and reconstructed the smooth muscle layers, which influenced the time of healing of the grafted vessels in vivo, reducing it considerably [40]. This information indicates the therapeutic potential of thymosin in medicine.

Role of Tβ4 in the Regulation of Inflammatory Response

It is postulated that Tβ4 is capable of inhibiting the inflammatory response. There are reports that it has an impact on the multi-stage activation of NFKB, the transcription factor which activates many pro-inflammatory genes [33]. Experiments were performed in which the inflammatory process was induced by the cytokine TNF-α and then the cells were treated with Tβ4. It turned out that a reduction occurred of nuclear NFKB amount, its activity and the degree of phosphorylation of its key subunit, p65. It is suggested that a block takes place in these cells of translocation of the p65 subunit to the nucleus [33]. There are also reports that Tβ4 sulfoxide, which is formed by monocytes in the presence of glucocorticoids, also inhibits inflammatory responses [20].

Tβ4 in the Functioning of the Heart

Of particular relevance to cardiovascular physiology is angiogenesis occurring during the development of the heart, when the outer layer of EPDC (epithelial derived cells) undergo transition from epithelial cells to mesenchymal and migrate into the deeper layers, the myocardium and subendocardium. After that, their differentiation into specific cell types occurs [27]. It also takes place under the influence of growth factors. Some of them, VEGF and bFGF (basic fibroblast growth factor), result
in differentiation into endothelial cells of coronary arteries. The second group, PDGF (platelet derived growth factor) and TGF β, causes transformation to smooth muscle cells which form the muscular layer of the veins and arteries of the heart.

It has been discovered that the role of Tβ4 in this process is the stimulating of progenitor cells to move into deeper layers of the heart. This fact is demonstrated by experiments with mice with a deletion mutation that causes a lack of cardiac expression of thymosin. In this case, EPDC undergo transitions to mesenchymal cells but do not have the ability to migrate and are trapped in the outer layer of the heart. Cells expressing α-SMA (α-smooth muscle actin) and endothelial specific receptor Tie2 have been identified on the surface of this organ, thus it can be concluded that the purpose of these cells was to transform into endothelial cells [27]. As a result, the coronary arteries are less numerous, tissue has abnormal structure and is poorly supplied with blood and nourished [27]. It was also discovered that Tβ4 may mobilize progenitor cells from the circulation, as well as from the epicardium [28]. The discovery of this mechanism may be important in the therapeutic strategies of cardiac diseases, in the regeneration of heart cells in patients after myocardial infarction or long-term ischemic disease. The assumption is that cell precursors may be used, avoiding the risk of transplanted stem cell rejection by the patient’s immune system [27].

**Thymosin as a Factor which Regulates Inhibition of Scarring**

It has been reported that Tβ4 positively influences healing by reducing scarring, inter alia, the heart after myocardial infarction. One of the experiments conducted on mice with artificially-induced myocardial infarction consisted of injecting Tβ4 once directly into the heart and in the form of several injections into the peritoneal area. The results were positive, the thymosin treated animals exhibited greater survival of myocardial cells and reduced scar formation by 53% at 4 weeks. The migration of progenitor cells from the inner layers of the heart and their differentiation into new coronary vessels was also found [28].

Tβ4 sulfoxide has also been shown to modulate scarring. It has been proven that Tβ4 sulfoxide stimulates wound healing and reduced scarring in mice after myocardial infarction [41]. It is known that it inhibits neutrophil chemotaxis and interferon-γ antiviral activity, and causes the dissipation of monocytes, which prevents the formation of foci of chronic inflammation and allows proper wound healing without the formation of non-functional scars [41].

**Therapeutic Potential of Tβ4**

A large number of researchers have postulated that thymosin β has great therapeutic potential. To test the safety of a formulation containing Tβ4, a series of experiments was done. On the basis of trials with transgenic mice, it appears that an excess (50 fold) of the protein did not result in adverse side effects in survival, but only had a negative impact on the development of dentition during fetal growth [28]. The tests performed with rats and dogs, which were administered endogenous Tβ4, showed no significant adverse effects of the protein. In the tests already carried out with humans, the results are promising, and provide evidence of the safety and efficacy in the therapeutic effect of Tβ4 use [28].

Similar hopes are associated with Ac-SDKP [19]. This peptide is so short that synthesizing it under laboratory conditions is relatively uncomplicated and not very expensive, which is an additional, unquestionable advantage. All these facts speak for the fact that Ac-SDKP is potentially a good pharmacological agent.

**Summary and Conclusions**

The analysis of the data makes it possible to conclude that thymosin β has a wide variety of functions in the body. Major activities which it exhibits are: actin binding, promotion of cell migration, angiogenesis, an anti-inflammatory and anti-apoptotic action, promoting wound healing, inhibition of fibrosis, stem cell recruitment and differentiation., protection from burns, cytotoxic agents and hypoxia [7, 24, 25, 27, 28, 30, 33]. It is worth noting that disturbances in the expression of this protein may have serious clinical consequences, particularly in the context of cancer [6, 36, 1, 23]. On the other hand, the experimental data supports the thesis about the potential therapeutic properties of Tβ4 and derivatives of this peptide-sulfoxide and Ac-SDKP. These molecules are considered to be a potential factor applicable in the treatment of cardiovascular diseases, corneal and skin wounds and brain injuries [19, 20, 22, 28]. Based on an understanding the numerous activities of Tβ4, it is postulated that the peptide will be used in the treatment of, inter alia, myocardial infarction, chronic heart failure, diabetes, lupus, stroke, multiple sclerosis, pressure ulcers, burns, dry eye, viral infections and septic shock [28]. Early clinical trials show Tβ4 to be safe, well tolerated and effective in dermal and eye wound healing. Similarly, clinical trials on the use of this peptide as a drug for heart disease are underway and expected to obtain promising results [28].
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References


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