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DIAGNOSTICS

Over-expression of thymosin β4 in granulomatous lung tissue with active pulmonary tuberculosis

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SUMMARY

Recent studies have shown that thymosin β4 (Tβ4) stimulates angiogenesis by inducing vascular endothelial growth factor (VEGF) expression and stabilizing hypoxia inducible factor-1α (HIF-1α) protein. Pulmonary tuberculosis (TB), a type of granulomatous disease, is accompanied by intense angiogenesis and VEGF levels have been reported to be elevated in serum or tissue inflamed by pulmonary tuberculosis.

We investigated the expression of Tβ4 in granulomatous lung tissues at various stages of active pulmonary tuberculosis, and we also examined the expression patterns of VEGF and HIF-1α to compare their Tβ4 expression patterns in patients' tissues and in the tissue microarray of TB patients.

Tβ4 was highly expressed in both granulomas and surrounding lymphocytes in nascent granulomatous lung tissue, but was expressed only surrounding tissues of necrotic or caseous necrotic regions. The expression pattern of HIF-1α was similar to that of Tβ4. VEGF was expressed in both granulomas and blood vessels surrounding granulomas. The expression pattern of VEGF co-localized with CD31 (platelet endothelial cell adhesion molecule, PECAM-1), a blood endothelial cell marker, and partially co-localized with Tβ4. However, the expression of Tβ4 did not co-localize with alveolar macrophages. Stained alveolar macrophages were present surrounding regions of granuloma highly expressing Tβ4.

We also analyzed mRNA expression in the sputum of 10 normal and 19 pulmonary TB patients. Expression of Tβ4 was significantly higher in patients with pulmonary tuberculosis than in normal controls.

These data suggest that Tβ4 is highly expressed in granulomatous lung tissue with active pulmonary TB and is associated with HIF-1α- and VEGF-mediated inflammation and angiogenesis. Furthermore, the expression of Tβ4 in the sputum of pulmonary tuberculosis patients can be used as a potential marker for diagnosis.

1. Introduction

Tuberculosis (TB) is one of the most important infectious pathogens worldwide and is a leading cause of death in humans [1]. The typical histological reaction to TB is necrotizing granulomatous inflammation accompanied by various types of non-necrotizing granulomas [2]. Granulomas provide a microenvironment in which antigen-specific T-cells co-locate with and activate infected macrophages that will inhibit the growth of *Mycobacterium tuberculosis*, the bacterium causing TB. Although the granuloma is the site of mycobacterial killing, virulent mycobacteria have developed a variety of mechanisms to resist macrophage-mediated death. These surviving mycobacteria become dormant if host cellular immunity or the signals maintaining granuloma structure wane, and if mycobacteria resume replication, this will lead to reactivation of TB. This balance of life and death applies not only to

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mycobacteria but also to the host's macrophages, which undergo apoptosis or necrosis, leading to the characteristic caseous necrosis within the granuloma and allowing for the potential reactivation of TB infection [3].

VEGF has been found to be associated with inflammatory diseases, and increased serum levels of VEGF are present in pulmonary TB [4–6]. Transcriptomic and proteomic approaches revealed that VEGF and its receptor, Flk-1, are up-regulated in macrophages infected with *M. tuberculosis* [7]. VEGF was also detected in pleural fluid and is known to correlate with inflammatory responses in tissue affected by TB [8]. A recent study shows that measurements of VEGF and HIF-1α in the pericardial fluid of malignant tissue had significantly more clinical value than these measurements in TB-infected tissue [9].

Thymosin β4 (Tβ4), a 43-amino acid actin-binding protein, has been reported to play key roles in inflammation, tumor growth, metastasis and angiogenesis [10–20]. Previous studies have shown that Tβ4 stimulates angiogenesis by induction of VEGF expression [10]. Additionally, the up-regulation of VEGF by Tβ4 is HIF-1α-dependent, and occurs by increasing the stability of HIF-1α protein in an oxygen-independent manner [12]. Immunohistochemical studies have shown that Tβ4 usually co-localizes with VEGF and HIF-1α and has been observed to be present in blood endothelial cells and lymphocytes [21,22]. Here, we analyzed the expression patterns of Tβ4 in granulomatous lung tissue at various stages of active pulmonary tuberculosis. We also compared the expression patterns of VEGF and HIF-1α and analyzed the expression patterns of Tβ4, VEGF and HIF-1α.

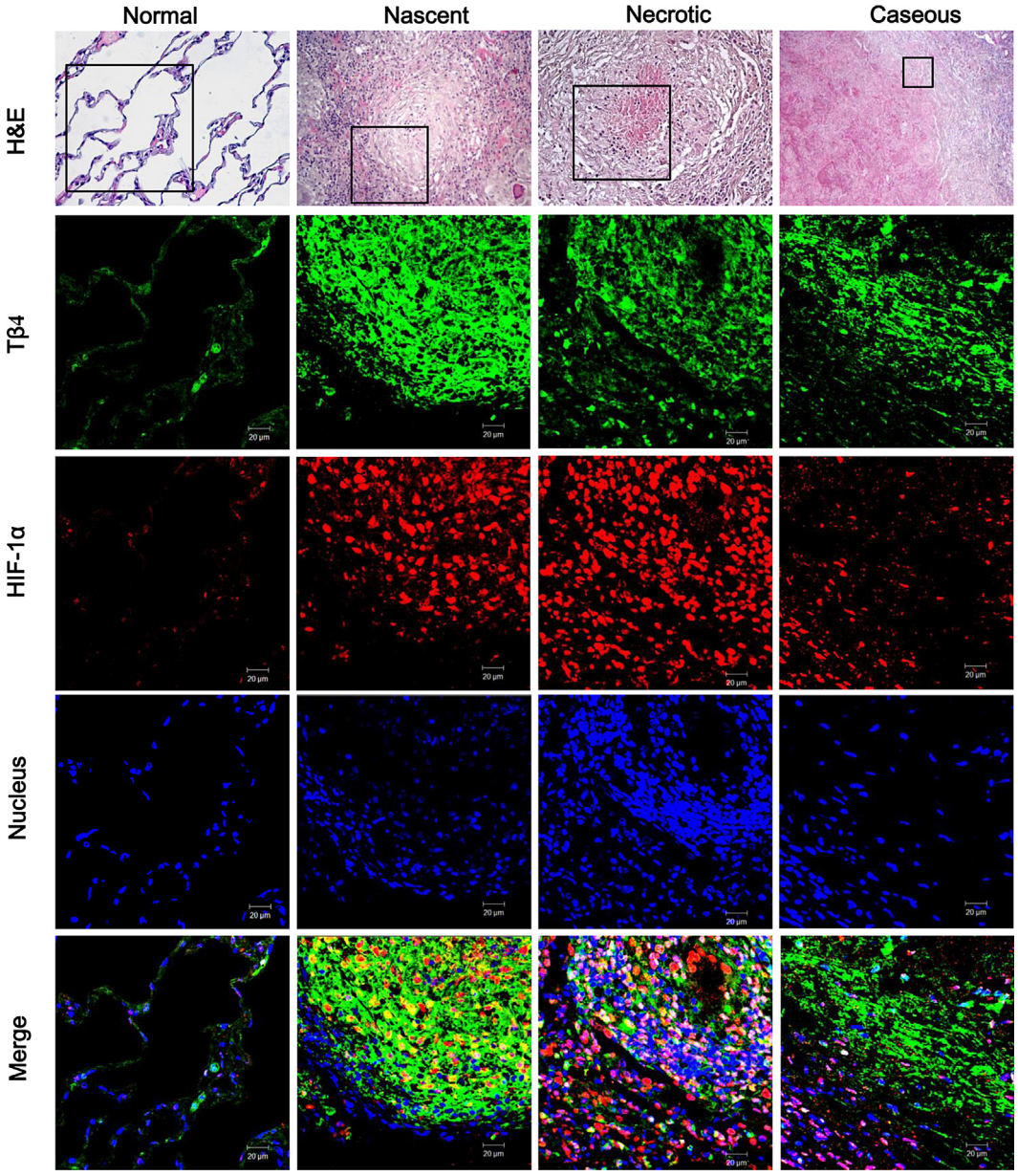

Figure 1. Expression and co-localization of Tβ4 and HIF-1α in normal, nascent, necrotic and caseous necrotic granulomatous lung tissue with active pulmonary tuberculosis. Tβ4 and HIF-1α were co-localized in the core of nascent and necrotic granulomas and the edge regions of caseous necrotic granulomas. Original magnification was X400 for immunofluorescence and X200 or 100 for H&E staining. Tβ4 was highly expressed in the core of granulomas in each stage and also expressed in the periphery of caseous necrotic granulomas. The expression pattern of HIF-1α was similar and co-localized with that of Tβ4 in nascent and necrotic granulomas but did not co-localize with Tβ4 in the periphery of caseous necrotic granulomas.