Non-canonical TGFβ signaling pathways in prostate cancer.

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Akademisk avhandling
som med vederbörligt tillstånd av Rektorsämbetet vid Umeå universitet för avläggande av medicine doktorsexamen vid medicinska fakulteten framläggs till offentligt försvaret i E04, Norrlands universitetssjukhus, Umeå, fredagen den 16 december, kl. 09:00.
Avhandlingen kommer att försvaras på engelska.

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Prostate cancer is the second leading cause of cancer-related death in men in the western world. Deregulation of transforming growth factor β (TGFβ) signaling pathway is frequently detected in prostate cancer and contributes to tumor growth, migration, and invasion. In normal tissue and the early stages of cancer, TGFβ acts as a tumor suppressor by regulating proliferation, differentiation, and apoptosis. In later stages of cancer, TGFβ acts as a tumor promoter by inducing angiogenesis, tumor invasion, and migration. Thus, it is important to investigate the molecular mechanisms behind the tumor-promoting effects of TGFβ, which is the topic of this thesis.

The tumor necrosis factor receptor–associated factor 6 (TRAF6) controls non-canonical TGFβ signals due to its enzymatic activity, causing polyubiquitination of the cell membrane–bound, serine/threonine kinase TGFβ type I receptor (TβRI) and its subsequent cleavage in the extracellular domain by tumor necrosis factor α–converting enzyme (TACE) in a protein kinase Cζ (PKCζ)-dependent manner. TRAF6 also recruits the active γ-secretase complex to the TβRI, resulting in a second cleavage in the transmembrane region and the liberation of the TβRI intracellular domain (TβRI-ICD), which enters the nucleus, where it associates with the transcriptional co-regulator p300. In Paper I, the aim was to elucidate by which mechanisms TβRI-ICD enters the nucleus. We found that the endocytic adaptor protein APPL1 interacts with TβRI and PKCζ. APPL proteins are required for TβRI translocation from endosomes to the nucleus via microtubules in a TRAF6-dependent manner. Moreover, APPL proteins are important for TGFβ-induced cell invasion, and high levels of APPL1 are detected by immunohistochemistry in prostate cancer. Finally, we demonstrated that the APPL1–TβRI complex visualized with the in situ proximity ligation assay (PLA) correlates with Gleason score, indicating that it might be a novel prognostic marker for aggressive prostate cancer. In Paper II, the aim was to explore by which mechanisms TGFβ causes activation of the AKT pathway, which regulates migration and therapy resistance of cancer cells. We found that the E7 ligase activity of TRAF6 induces Lys63-linked polyubiquitination of p85α upon TGFβ stimulation, resulting in plasma membrane recruitment, Lys63-linked polyubiquitination, and subsequent activation of AKT. Moreover, the TRAF6 and PI3K/AKT pathway were found to be crucial for the TGFβ-induced migration. Importantly, we demonstrated, by PLA, a correlation between Lys63-linked polyubiquitination of p85α and aggressive prostate cancer in tissue sections from patients with prostate cancer. In Paper III, the aim was to investigate the mechanisms for TGFβ-induced activation of PKCζ and the role of PKCζ in tumor regression. We found that TRAF6 caused Lys63-linked polyubiquitination of PKCζ. By using two novel chemical compounds that inhibit PKCζ, we demonstrated that PKCζ is crucial for prostate cancer cell survival and invasion. In Paper IV, the aim was to investigate further the target genes for the nuclear TβRI-ICD–APPL1 complex identified in Paper I. We provide evidence that APPL proteins and the TGFβ signaling pathway are important for cell proliferation. TβRI regulates cell mitosis and cytokinesis by binding to AURKA in the centrosome and AURKB in the midbody. APPL1 also interacts with AURKB and survivin. TβRI kinase inhibitor suppresses the activation of AURKA and AURKB. In summary, the results reported in this thesis suggest the potential usefulness of the identified signaling components of the tumor-promoting effects of TGFβ as drug targets and biomarkers for aggressive prostate cancer.

Keywords: TGFβ, TβRI, TRAF6, APPL1, ubiquitination, AKT, p85α, PKCζ, AURKA, AURKB, midbody, mitosis, cytokinesis, invasion.