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Oncostatin M induces tumorigenic properties in non-transformed human prostate epithelial cells, in part through activation of signal transducer and activator of transcription 3 (STAT3)

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ABSTRACT

Prostate cancer is one of the most common types of cancer in men in Western countries. Chronic inflammation in the prostate, regulated by a complex network of factors including inflammatory cytokines, is one of the established risk factors for development of prostate cancer. Interleukin-6 (IL-6) is a well-known promoter of inflammation-induced carcinogenesis and disease progression in prostate cancer. Presence in the prostate and possible roles in tumor development by other members of the IL-6 family of cytokines have, however, been less studied. Here we show that the IL-6-type cytokine oncostatin M (OSM) indeed induce cellular properties associated with tumorigenesis and disease progression in non-transformed human prostate epithelial cells, including morphological changes, epithelial-to-mesenchymal transition (EMT), enhanced migration and pro-invasive growth patterns. The effects by OSM were partly mediated by activation of signal transducer and activator of transcription 3 (STAT3), a transcription factor established as driver of cancer progression and treatment resistance in numerous types of cancer. The findings presented here further consolidate IL-6-type cytokines and STAT3 as promising future treatment targets for prostate cancer.

1. Introduction

For cancer to occur, the normal cell has to proceed through a complex array of events commonly denoted 'the hallmarks of cancer', involving both genotypic and phenotypic cellular changes [1]. Early clinical signs of development of solid tumors involve aberrant cell growth, detected as abnormal properties of both cells and tissues. In many cases the cells also undergo changes in differentiation state over time, which in turn results in generation of cellular traits that further drives the neoplastic development. For example, tumors of epithelial origin that are driven to proceed through the epithelial-to-mesenchymal transition (EMT) process, will obtain a mesenchymal phenotype over time resulting in cells with several cellular properties that contribute to disease development and progression [2]. One such property is the ability to migrate, enabling invasion into surrounding tissues, dissemination and, in many cases, also subsequent metastatic establishment in secondary sites. The neoplastic development is orchestrated by a complex network of cell types in the pre-tumorigenic site, including both resident cell types belonging to the stromal compartment of the tissue, as well as cells recruited from distant sites, including specific subgroups of immune cells. Together these cell types constitute the tumor microenvironment, and their communication will result in both pro-tumorigenic and anti-tumorigenic signals regulating disease initiation and progression.

Prostate cancer is the second most common cancer type in men worldwide, with approximately 165,000 new cases and over 29,000 deaths estimated to occur during 2018 in the Unites States alone, according to statistics by the American Cancer Society [3]. In contrast to many other solid tumors, tumors in the prostate are not primarily established due to genotypic changes. Instead, long-term changes in the prostate microenvironment are believed to be one main reason for neoplastic initiation. One suggested risk factor is the presence of chronic, asymptomatic inflammation that is very common in the prostate of adult men [4]. In a recently reported prospective study, inflammation in benign prostate tissue was indeed shown to be a prognostic factor for later development of prostate cancer [5].

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The inflammatory milieu contains a large pool of signaling factors that both contribute to the persistence of the inflammatory state as well as regulating tumorigenesis and cancer progression. Pro-inflammatory cytokines are important inducers and mediators of inflammatory states, and the interleukin-6 (IL-6) family is one of the most studied families of inflammatory cytokines. The IL-6-type cytokines all bind to membrane-bound receptors and exert their effects through signal transduction via a common glycoprotein, gp130. Intracellular signaling is subsequently mediated by several pathways, including activation of both Janus kinases (JAKs) and signal transducers and activators of transcription (STATs), as well as mitogen-activated protein (MAP) kinases. Specifically, aberrant activation of the transcription factor STAT3 has been shown to play an important role in tumor initiation and disease progression in many types of cancer [6,7].

In prostate cancer, IL-6 has been shown to be a potent mediator of tumorigenesis as a major player in inflammatory sites in the prostate [8]. Immunohistochemical analysis comparing expression in normal prostate tissue and prostate cancer tissue of ligands and receptors belonging to the IL-6 family has identified differences in expression patterns in different disease stages. In normal prostate tissue, IL-6 is mainly expressed in epithelial cells, whereas leukemia inhibitory factor (LIF) and oncostatin M (OSM), two other cytokines belonging to the IL-6 family, are more highly expressed within the stromal compartment of the prostate [9]. Interestingly, the expression of all three cytokines increased in tissue with both benign prostatic hyperplasia (BPH) and prostate cancer compared to normal prostate, and all three factors were also more ubiquitously expressed in epithelial cells and stroma. The exclusive expression of OSM in prostate epithelium in cases with BPH or cancer, compared to the absence of OSM expression in epithelial cells in normal prostate tissue, suggested OSM as a putative marker for diagnostic evaluation of prostate tissue [9].

Although IL-6 has been established as a driver of carcinogenesis and tumor progression in the prostate, less is known about the possible roles of other IL-6-type cytokines in prostate cancer initiation and progression. Here we show that OSM indeed induce protumorigenic properties in normal prostate epithelial cells, further establishing the importance of IL-6-type cytokine signaling in prostate cancer initiation and progression.

2. Materials and methods

Keratinocyte serum-free cell culture medium (K-SFM), recombinant human epidermal growth factor (EGF), bovine pituitary extract (BPE), gentamicin, Cell Extraction Buffer, ProLong Gold antifade reagent with DAPI, NuPAGE LDS Sample Buffer, NuPAGE Sample Reducing Agent, NuPAGE Antioxidant, NuPAGE 4–12% Bis-Tris Gels, NuPAGE MOPS SDS Running Buffer were purchased from Life Technologies/Gibco (Paisley, UK). Recombinant human interleukin-6, recombinant human Oncostatin M, Human EMT 3-Color Immunocytochemistry kit, and StemXVivo serum-free tumorsphere media were obtained from R&D Systems (Abingdon, UK). Normal donkey serum was purchased from Dako (Glostrup, Denmark). The small molecule inhibitor of STAT3, Stattic, was purchased from Sigma-Aldrich (Stockholm, Sweden). Protease Inhibitor Cocktail Tablets were obtained from Roche (Stockholm, Sweden). High performance glass cover slips were purchased from Zeiss (Oberkochen, Germany). Glass bottom 12-well plates were obtained from MatTek (Ashland, MA, USA). TransBlot Turbo Transfer System, TransBlot Turbo Mini-size Nitrocellulose membranes, Trans-Blot Turbo Mini-size Transfer Stacks and Trans-Blot Turbo 5× Transfer Buffer were purchased from Bio-Rad (Stockholm, Sweden). Secondary goat anti-mouse IRDye 680RD and goat-anti rabbit IRDye 800 antibodies and Odyssey Blocking Buffer were obtained from LI-COR (Cambridge, UK). Anti-p-STAT3 antibody (pY705, ab76315) and anti-Actin antibody (ab3280) were purchased from Abcam (Cambridge, UK).

2.1. Cell culture

Non-transformed human prostate epithelial cells from the RWPE-1 cell line (ATCC/LGC Standards, Manassas, VA, USA) were cultured in serum-free keratinocyte cell culture media (K-SFM) supplemented with human EGF (5 ng/mL), bovine pituitary extract (25 μg/mL), and gentamicin (50 μg/mL). For evaluation of effects by IL-6-type cytokines, cells were incubated in the absence (control) or presence of either IL-6 or OSM (both at 100 ng/mL) for different periods of time.

2.2. Evaluation of cell size, cell number and epithelial-to-mesenchymal transition (EMT) by immunocytochemistry

For evaluation of cell size, cell number and EMT, RWPE-1 cells were seeded on glass coverslips in 6-well plates at a density of 2×10^4 cells per well and cultured in the absence (control) or presence of test substance for 5 or 8 days before analysis of cell properties. After the culture period, cells were fixed with 4% PFA for 10 min in 37 °C followed by blocking in 0,3% Triton X-100, 1% BSA, and 10% normal donkey serum in PBS for one hour. Evaluation of differentiation status of the cells was performed by staining with antibodies against the EMT markers E-cadherin, Snail and vimentin using an EMT 3-Color Immunocytochemistry kit according to the instructions by the manufacturer. Cells were mounted using ProLong Gold anti-fade reagent with DAPI before images were generated using a Zeiss LSM710 confocal microscope. Analysis of cell volume, number of cells per image and cellular staining intensity mean of the different EMT markers was performed in at least 120 cells per treatment condition using the IMARIS (Bitplane) software.

2.3. Tracking of cell migration using live cell imaging

Cell migration was evaluated using live cell imaging of RWPE-1 cells, 1×10^5 cells were seeded in glass bottom 12-well plates and cultured in the absence (control) or presence of test substance for five days. After the initial five-day culture, nuclear staining was carried out by addition of Hoechst 33342 dye (0.5 μg/mL) to the cell cultures immediately before imaging. During live cell imaging cells were cultured in 37 °C and 5% CO_2 in the absence or presence of test substance, and images were generated every sixth minute for 450 min with multiple tile scans using a Zeiss LSM710 confocal microscope. Automatic tracking of cell migration of at least 2000 cells was performed using the IMARIS software.

2.4. Immunoblotting

For Western blot studies on activation by phosphorylation of STAT3, RWPE-1 cells were seeded in 6-well plates at a density of 3×10^5 cells per well. After attachment overnight, the cells were incubated with or without test substance for different periods of time (5–60 min) before incubation was terminated by washing with ice-cold PBS. Cell lysis was performed using Cell Extraction Buffer complemented with 1× Complete Protease Inhibitor Cocktail and 1 mM PMSF. Cell lysates were then centrifuged at 21 500× g for 10 min at 4 °C, supernatants were collected and stored at −80 °C. For Western blot, cell lysates were electrophoretically separated and subsequent transfer was performed using a TransBlot Turbo Transfer System (Bio-Rad). Membranes were blocked for one hour using Odyssey Blocking buffer in PBS (1:4) at room temperature