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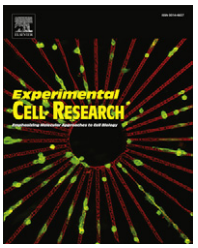

Research Article

Curcumin targets fibroblast–tumor cell interactions in oral squamous cell carcinoma

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ABSTRACT

Co-culture of periodontal ligament fibroblasts (PDLs) and SCC-25 oral squamous carcinoma cells (OSCC) results in conversion of PDLs into carcinoma-associated fibroblasts (CAFs) and induces epithelial-to mesenchymal transition (EMT) of OSCC tumor cells. We hypothesized that Curcumin targets this dynamic mutual interaction between CAFs and tumor cells. Normal and 2 μM Curcumin-treated co-culture were performed for 4 days, followed by analysis of tumor cell invasivity, mRNA/protein expression of EMT-markers and mediators, activity measure of matrix metalloproteinase 9 (MMP-9), and western blot analysis of signal transduction in tumor cells and fibroblasts. In Curcumin-treated co-culture, in tumor cells, the levels of nuclear factor κB (NFκBα) and early response kinase (ERK)—decreased, in fibroblasts, integrin αv protein synthesis decreased compared to corresponding cells in normal co-culture. The signal modulatory changes induced by Curcumin caused decreased release of EMT-mediators in CAFs and reversal of EMT in tumor cells, which was associated with decreased invasion. These data confirm the palliative potential of Curcumin in clinical application.

Introduction

Recent data suggest a pivotal role of collaborative interactions between neoplastic cancer cells and their supporting stroma in tumor growth, progression and treatment resilience of most cancers [1]. In this study, we focus on cells of head and neck squamous cell carcinoma (HNSCC), a common cancer form in men. Our recent investigations indicate that invasive HNSCC

Abbreviations: AKT, another kinase of transcription; AP-1, activator protein-1; bcl2, B-cell lymphoma-2; BDNF, brain-derived neurotrophic factor; CAFs, carcinoma-associated fibroblasts; COX-2, prostaglandin-endoperoxide synthase 2; DMSO, dimethyl sulfoxide; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; ERK, early response kinase; FBS, fetal bovine serum; HNSCC, head and neck squamous cell carcinoma; IL, interleukin; ITGA5, integrin αV; MMP, matrix metalloproteinase; MTT, 3-(4,5-dimethylthiazol-2-yl)2,5diphenyltetrazolium bromide; NFkBα, nuclear factor kBα; OSCC, oral squamous cell carcinoma; PDLs, periodontal ligament (PDL) fibroblasts; TGF-β1, transforming growth factor-β1; TrkB, neurothrophin receptor B

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tumor cells undergo epithelial-to-mesenchymal transition (EMT), which leads to gain of mesenchymal characteristics in epithelial cells. This enables them to migrate across the basal membrane and initiate metastasis formation [2–4]. This phenotype of tumor cells is a consequence of a communication between tumor cells and carcinoma associated fibroblasts (CAFs), which is orchestrated by cytokine–chemokine interactions [2,3,5].

In a co-culture model of periodontal ligament fibroblasts (PDLs) and SCC-25 oral squamous carcinoma cells (OSCC), we investigated the conversion of normal fibroblasts into CAFs and EMT in SCC-25 cells [2]. We observed that SCC-25 cells produce active, processed IL-1β, and PDLs possess receptor for it, whose expression is increased in the presence of SCC-25 tumor cells. Upon interaction with SCC-25 cells active IL-1β signaling occurs in co-cultured fibroblasts leading to induction of several genes involved in tumor progression, including interleukin-6 (IL-6) and prostaglandin-endoperoxide synthase 2 (COX-2) [3]. In fact, dynamic interaction between CAFs and tumor cells dictate gene expression changes in the interacting cells, which covers major events of tumor progression. We have previously reported that fibroblasts might facilitate the invasion of SCC-25 cells by expressing matrix metalloproteinases (MMPs) on their own, in response to tumor-cell-produced cytokines (i. e. IL-1β), to TGF-β)1 or to integrin–fibronectin interactions [5]. At the same time, the tumor cells are capable of activating pro-MMPs [5]. For instance pro-MMP-2 is higher expressed in fibroblasts, but its activation only occurred in the presence of tumor cells. In contrast to pro-MMP-2, pro-MMP-9 is produced by SCC-25 cells, not by PDLs, and pro-MMP-9 is activated on the surface of tumor cells. In fact, the paracrine interaction between CAFs and tumor cells is required for the upregulation of MMP-9 [5]. A dynamic mutual interaction of CAFs and tumor cells is a prerequisite for tumor progression and it represents a significant therapeutic target.

Curcumin (diferuloylmethane) is a polyphenol derived from the *Curcuma longa* plant, commonly known as turmeric. Curcumin has been used extensively in Ayurvedic medicine for centuries, as it is nontoxic and has a variety of therapeutic properties including anti-oxidant, analgesic, anti-inflammatory and antiseptic activity. Curcumin has been found to possess anticancer activities via its effect on a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis. Curcumin has shown anti-proliferative effect in multiple cancers, and is an inhibitor of the transcription factor NFκBα and downstream gene products (including c-Myc, Bcl-2, COX-2, nitric oxide synthase (NOS), Cyclin D1, tumor necrosis factor alpha (TNF-α), interleukins and MMP-9) [6]. Studies of Curcumin in various head and neck cancer cell lines have demonstrated decreased cell growth and survival, which was related with inhibition of NFκBα activation [7].

The lipophilic nature of Curcumin and relative insolubility in aqueous solutions, combined with short half life and low bioavailability following oral administration has presented a significant challenge in developing an effective delivery system for its use as a chemotherapeutic agent [8]. In an effort to overcome this obstacle, various strategies are being tried including the development of liposomal, phospholipid and nanoparticulated formulations of the compound to enable intravenous administration [8]. In a pharmacokinetic study performed in rats, following oral administration of the Curcumin–phosphatidyl choline complex, the maximal achieved plasma concentration of Curcumin was around 800 ng/ml [9], which is 2.17 μmol/L. In a human phase 1 trial Curcumin was orally applied for advanced colorectal cancer, patients consumed 3.6 g of Curcumin daily, and up to 2–2.5 μM Curcumin concentrations have been detected in their urine. Temporary stable diseases have been observed in two patients, whereas no response was observed in the other study participants [10]. In an *in vitro* study: Curcumin above 50 μmol/L concentration caused growth suppression of HNSCC cell lines [11], which is much higher than the concentrations achievable *in vivo*.

In the current study we hypothesize that Curcumin targets the dynamic mutual interaction of CAFs and tumor cells in head and neck cancer. We assume that Curcumin achieves this effect even at those low concentration levels that are detected in blood or urine (2 μmol/L) in previous *in vivo* studies. We presume that it modifies the interaction between carcinoma associated fibroblasts and tumor cells, which leads to decrease of the tumor cell invasivity, without growth suppression effects on tumor cells.

Materials and methods

Chemicals

Curcumin powder was purchased from Sigma-Aldrich (Vienna, Austria). Curcumin is a lipophilic polyphenol and thus is insoluble in water, but is readily soluble in organic solvents such as dimethyl sulfoxide (DMSO), acetone and ethanol [12,13]. A stock solution of Curcumin was prepared at 100 mmol/L in DMSO. Chemicals used in the study were purchased from Sigma, from Roth (Karlsruhe, Germany) and from Serva (Heidelberg, Germany).

Cell lines

PDL fibroblasts [2,14] were isolated from periodontal ligament (PDL) and received from Prof. Dr. Miosge (Department of Prosthodontics, Georg-August-University, Göttingen, Germany) [14]. They were routinely cultured in DMEM-low glucose (PAA, Pasching, Austria) supplemented with 10% fetal bovine serum (FBS) (PAA), 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin. SCC-25 cells were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany, ref. ACC 617), and were routinely cultured in DMEM/F12 (PAA) supplemented with 10% FBS (PAA), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, 100 μg/ml streptomycin [2,3,5,15]. BEAS-2B immortalized bronchial epithelial cells [15,16] were purchased from the European Collection Agency of Cell Cultures (Salisbury, UK), were routinely cultured in RPMI-1640 (PAA) supplemented with 10% fetal bovine serum (FBS) (PAA), 2 mM L-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin.

Treatment of cells with Curcumin

The Curcumin treatment of the cells is based on a published Ref. [11]. SCC-25 cells, PDL fibroblasts and BEAS-2B immortalized epithelial cells were plated in 96-well plates, with 10^4 cells per