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Effects of cigarette smoke condensate on oral squamous cell carcinoma cells

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ABSTRACT

Objective: Epidemiological studies have reported that tobacco use is a major etiological factor for oral cancer. Several matrix metalloproteinases (MMPs) have been shown to play important roles in the invasion and metastasis of oral squamous cell carcinomas, especially MMP-2 and MMP-9. This study examined the effects of cigarette smoke condensate (CSC) on oral cancer cells.

Design: Two oral squamous cell carcinoma cell lines, SCC-25 (metastatic) and CAL-27 (non-metastatic), were exposed to different concentrations of CSC and examined for their collagen degrading ability and MMP production using collagen degradation assays, zymograms and Western blots.

Results: Exposure to CSC increased the collagen degrading ability of the metastasizing cell line (SCC-25) by a mechanism involving increased MMP-2 and MMP-9 production.

Conclusion: CSC increased the collagen degrading ability of SCC-25 by increasing the MMP-2 and MMP-9 protein levels. Continued cigarette smoking in oral cancer patients may result in decreased survival rates due to enhanced metastatic potential of the cancer cells.

1. Introduction

Oral cancer is the sixth most common malignancy worldwide with 90% of oral malignancies being oral squamous cell carcinomas (SCC).[1] The specific molecular events involved in the carcinogenesis process, in addition to the sequence in which they usually occur, are still not clearly defined.[2] Although most epidemiological studies disclosed a significant association between oral SCC and the smoking habit of the patients, the specific targets of these tobacco agents and the exact nature of their association with SCC remain largely unknown.

Tumour cell invasion and metastasis are complex processes that involve both tumour cell and host cell activities. One of the key elements in the invasion and metastatic cascade involves the disruption of extracellular matrix (ECM) and basement membranes that permits tumour cells to access the surrounding structures and the distant metastatic sites.[3] The interaction between ECM proteins and tumour cells is an important initial step in the invasion process. This interaction is a poorly understood mechanism, but it certainly involves the matrix metalloproteinases (MMPs).[4]

The MMPs are a family of zinc dependent proteinases, which are secreted as proenzyme (latent enzyme) and require proteolytic cleavage for activation. Oral SCC cells produce several MMPs, which include MMP-1, MMP-2, MMP-7, and MMP-9.[5–7] MMP-1 initiates the degradation of type I collagen, which is one of the most abundant proteins of the ECM. Once

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cleaved, type I collagen denatures into gelatin and the ultimate degradation of gelatin is accomplished by the action of the gelatinases (MMP-2 and MMP-9).[5] Another essential step in the invasion and metastasis of tumour cells is the degradation of type IV collagen in the basement membrane by MMP-2 and MMP-9.[8] MMP-2 and MMP-9 are known to be closely associated with the malignant potential of tumour cells.[6]

Cigarette smoke is a complex mixture that contains over 4000 different compounds.[9] Eighty-one of these compounds have been classified as carcinogens by the International Agency for Research on Cancer (IARC). They were defined as being pharmacologically active, toxic, mutagenic and carcinogenic in nature.[10,11] Cigarette smoke condensate (CSC) has been used as a surrogate for cigarette smoke in most experimental studies and is a highly genotoxic substance that is capable of causing various types of DNA damage.[9]

The association between cigarette smoke as a causative factor for the development of oral cancer is well documented,[12,13] whilst its effects on already established oral cancer cells have not been elucidated. The purpose of this study was to gain insights into the mechanisms by which cigarette smoke could affect the behaviour of oral cancer cells. Two OSCC cell lines, SCC-25 and CAL-27, were exposed to different concentrations of CSC and examined for their collagen degrading ability and MMP production. The results demonstrated that the exposure to CSC increases the collagen degrading ability by a mechanism involving increased MMP-2 and MMP-9 production from the metastasizing SCC-25 cell line.

2. Materials and methods

2.1. Cell culture

The SCC-25 (CRL-1628), and CAL-27 (CRL-2095) cell lines were purchased from American Type Culture Collection (Manassas, VA). The cell lines were derived from metastasizing (SCC-25) and non-metastasizing (CAL-27) SCC tongue lesions. The cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicillin, 50 μg/ml gentamycin, and 2.5 μg/ml fungizone at 37 °C in 5% CO_2 in air. Each cell line was used within 7–15 passages after initiation of cultures from American Type Culture Collection.

2.2. Measurement of cellular proliferation by water-soluble tetrazolium-1 (WST-1) assay

Mitochondrial dehydrogenase activities were determined utilising the WST-1 assay (Roche Applied Science, Indianapolis, IN). The principle of the assay is based on the fact that the tetrazolium salts are cleaved to formazan by the mitochondrial dehydrogenases. An expansion in the number of viable cells results in an increase in the overall activity of mitochondrial dehydrogenases in the sample. This augmentation in enzyme activity leads to an increase in the amount of formazan dye formed, which directly correlates to the number of metabolically active cells in the culture. The SCC-25 and CAL-27 cells were detached with 0.25% trypsin, pelleted, resuspended in fresh media, and seeded as (60,000 cells/well) in 6-well plates with 2 ml DMEM supplemented with 10% FBS. The plates were incubated for 24 h to allow the cells to attach, the media was then removed and the cells were exposed to 1 ml of various particulate matter concentrations (400, 200, 100, 50, 25, and 0 μg/ml) of the cigarette smoke condensate (Murty Pharmaceuticals, Lexington, KY) diluted in serum free media. After 72 h, the media was removed from the 6-well plates and the cell proliferation reagent WST-1 (100 μl WST-1 and 900 μl media) was added and the plate incubated for 0.5, 1, and 2 h at 37 °C and 5% CO_2. A 100 μl sample from each well was placed in a 96-well plate and the absorbance of the samples against the negative control (media without cells) as the blank was measured using a microplate reader (Titertek, Flow laboratories, Mclean, VA) at 450 nm. The average of three readings was calculated and the absorbance values of each sample were compared with the positive control (untreated cells) by percentage according to the equation:

$$\text{cell proliferation}(\%) = \frac{(\text{experiment value} - \text{negative control})}{(\text{positive control} - \text{negative control})} \times 100\%$$

2.3. Measurement of cellular cytotoxicity by lactate dehydrogenase (LDH) assay

Cell toxicity was assessed by using the Cytotoxicity Detection Kit Plus (Roche Applied Science, Mannheim, Germany). The principle of this assay is the quantification of cell death and cell lysis. It is based on the measurement of lactate dehydrogenase activity released from the cytosol of damaged cells. Cells were treated with the same range of concentrations of CSC used in the WST-1 assays for 72 h. The high control (total cell death) was generated by adding 1.9 ml of media and 100 μl lysis solution provided by the manufacturer to the control cells after 72 h, which gave the maximum release of LDH. The low control consisted of media from untreated control cells after 72 h, which gave the minimal release of LDH. Media without cells was utilized as the background level of the assays. After 72 h, media from each of the wells was transferred to 96 well plates and 100 μl of reconstituted mix as per the manufacturer was added to each well. The plates were then incubated for 15 min at room temperature. Absorbance was recorded using a microplate reader (Titertek, Flow laboratories, Mclean, VA) at 490 nm. The percentage release of LDH from the treated cells was calculated by comparing it to the maximum release of LDH achieved by the lysis solution used on the control cells. To determine the cytotoxicity, the absorbance values of the background were subtracted from that of the experimental samples and the cytotoxicity was calculated according to the equation:

$$\text{cytotoxicity}(\%) = \frac{(\text{experiment value} - \text{low control})}{(\text{high control} - \text{low control})} \times 100\%$$

2.4. Collagen degradation assay

Six-well culture plates were coated with a film of reconstituted type I collagen as previously described.[12] Briefly, a stock