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Inhibition of nicotinamide phosphoribosyltransferase and depletion of nicotinamide adenine dinucleotide contribute to arsenic trioxide suppression of oral squamous cell carcinoma

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ABSTRACT

Emerging evidence suggests that increased nicotinamide phosphoribosyltransferase (NAMPT) expression is associated with the development and prognosis of many cancers, but it remains unknown regarding its role in oral squamous cell carcinoma (OSCC). In the present study, the results from tissue microarray showed that NAMPT was overexpressed in OSCC patients and its expression level was directly correlated with differential grades of cancer. Interestingly, treatment of OSCC cells with chemotherapy agent arsenic trioxide (ATO) decreased the levels of NAMPT protein and increased cellular death in an ATO dose- and time-dependent manner. Most importantly, combination of low concentration ATO with FK866 (a NAMPT inhibitor) exerted enhanced inhibitive effect on NAMPT protein and mRNA expressions, leading to synergistic cytotoxicity on cancer cells through increasing cell apoptosis and depleting intracellular nicotinamide adenine dinucleotide levels. These findings demonstrate the crucial role of NAMPT in the prognosis of OSCC and reveal inhibition of NAMPT as a novel mechanism of ATO in suppressing cancer cell growth. Our results suggest that ATO can significantly enhance therapeutic efficacy of NAMPT inhibitor, and combined treatment may be a novel and effective therapeutic strategy for OSCC patients.

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1. Introduction

Oral squamous cell carcinoma (OSCC) is a common malignant tumor, which most frequently occurs on the tongue. Despite advances in therapeutic methods, including surgery in combination with radiation and/or chemotherapy, the survival rate of this disease has not been significantly increased from 50–60% in past decades (Rivera, 2015). Therefore, novel and more effective therapeutic strategies for patients with OSCC are urgently needed.

Nicotinamide phosphoribosyltransferase (NAMPT), also named pre-B-cell colony-enhancing factor (PBEF) or visfatin, was originally thought to be a cytokine that functions as a co-factor for B cell maturation (Samal et al., 1994). It was later identified as a crucial enzyme involved in nicotinamide adenine dinucleotide (NAD) biosynthesis (Rongvaux et al., 2002). NAD is both an essential coenzyme involved in cellular redox reactions and a substrate for NAD-dependent enzymes such as PARP and Sirtuins (Garten et al., 2015). Cancer cells have a high capacity for glucose uptake and an increased rate of glycolysis even in the presence of oxygen, called the Warburg effect (Warburg, 1956). This high metabolic demand requires increased NAD, which is involved in many critical processes for cancer including transcriptional regulation, cell-cycle progression, anti-apoptosis, and DNA repair (Chiarugi et al., 2012). Since NAD is rapidly consumed by NAD-dependent enzymes, NAMPT is critically important for the replenishment of the intracellular NAD pool in cancer cells (Hasmann & Schemainda, 2003). Recently, it has been reported that NAMPT plays a pivotal role in malignant tumors. The development of many cancers is associated with increased NAMPT expression (Galli et al., 2013). Furthermore, higher NAMPT expression correlates with increased tumor invasion, metastatic potential, and chemotherapy resistance in some malignancies (Vora et al., 2016). NAMPT inhibitors have been evaluated in a variety of tumors, both *in vitro* and in nude-mouse xenografts, in which the inhibitors were shown to be able to reduce tumor growth (Bi et al., 2011; Gehrke et al., 2014; Soncini et al., 2014). However, the pathological role of NAMPT in OSCC remains unknown.

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Arsenic trioxide (ATO) is a chemotherapy agent that has been successfully used to treat patients with acute promyelocytic leukemia (Shen et al., 1997). Accumulating evidences from *in vitro* studies indicate that ATO is also a promising therapeutic drug for certain solid malignant tumors (Uslu et al., 2000). The anti-cancer molecular mechanisms of ATO have been suggested to include inducing apoptosis, promoting differentiation, activating ROS generation, and inhibiting the mitochondrial permeability (Chen et al., 1996, 1998; Zhu et al., 1999). However, the effect and mechanism of ATO on NAMPT expression in cancer remain unclear.

In the present study, we investigated the expressions of NAMPT in three differentiated grades of OSCC in patients, and studied the effects of ATO on NAMPT in OSCC cell lines and its relevant mechanisms.

2. Materials and methods

2.1. Tissue microarray and immunohistochemistry

The specimens of OSCC for tissue microarray (TMA) analysis were purchased from Biomax US (Rockville, MD), catalog numbers OR601a. The OR601a consists of 61 tissue samples, which contains 50 cases of OSCC and 10 cases of normal and adjacent tissue (NAT), and its details of OSCC clinical characteristics are as following: age, ≤45, 14 cases; >45, 46 cases; gender, male, 37 cases; female, 23 cases; TNM grading, stage I, 20 cases; stage II, 24 cases; stage III, 5 cases; stage IV, 1 case (the TNM classification is a cancer staging notation system with alphanumeric codes. T, the size of the original tumor and whether it has invaded nearby tissue; N, regional lymph nodes that are involved; M, distant metastasis.); histopathologic differentiation, well-, 37 cases; moderately-, 8 cases; poorly-, 5 cases.

Immunohistochemistry was performed on all specimens to examine the NAMPT protein expression. In brief, the specimens were stained with goat anti-rat antibody against NAMPT (1:100, Santa Cruz Biotech, Santa Cruz, CA) by routine immunohistochemical assay. NAMPT immune staining was quantified and analyzed with an Image-Pro Plus 6.0 in 10 different fields in each section.

2.2. Cell culture

SCC15 and SCC25 cells were obtained from American Type Culture Collection (Manassas, VA), and were cultured in a 1:1 mixture of DMEM and Ham's F12 medium (Thermo Fisher Scientific, Waltham, MA) with 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, MA), 100 IU/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

2.3. PrestoBlue cell viability assay

After incubation with varying concentrations of ATO (1, 2, 4, and 8 μM) or FK866 (NAMPT inhibitor, 10 nM), survival rate of SCC15 and SCC25 cells was measured using PrestoBlue™ cell viability reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The $OD_{570/600}$ was recorded on a SpectraMAX 190 microplate reader (Molecular Devices, Sunnyvale, CA).

2.4. Cell immunofluorescence

For immunofluorescence staining, SCC15 and SCC25 cells were cultured in a glass bottom cell culture dish (Nest, Wuxi, China). After treatment with varying concentrations of ATO (1, 2, 4, and 8 μM) or/and FK866 (10 nM) for 48 h, the cells were incubated with NAMPT antibody (1:50) at 37 °C for 1 h and 4 °C overnight, and followed by Cy3-labeled secondary antibody (Thermo Fisher Scientific, Waltham, MA) for 1 h. The cells were then counterstained with Hoechst (Thermo Fisher Scientific, Waltham, MA). Images were subsequently captured using an inverted fluorescence microscope (Leica DMi3000 B, Mannheim, Germany). The positive staining was shown as red fluorescence.

2.5. TUNEL assay

To evaluate apoptosis induced by ATO and FK866, SCC15 and SCC25 cells were stained with terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end labelling (TUNEL, In Situ Cell Death Detection Kit, Roche, Penzberg, Germany). The detection procedure was performed according to the manufacturer's instructions. OSCC cells were observed using an inverted fluorescence microscope (Leica DMi3000 B, Mannheim, Germany). The nuclei of apoptotic cells were stained green fluorescence with FITC and quantitated by counting the number of TUNEL-positive cells in 10 random microscopic fields at ×400 magnification.

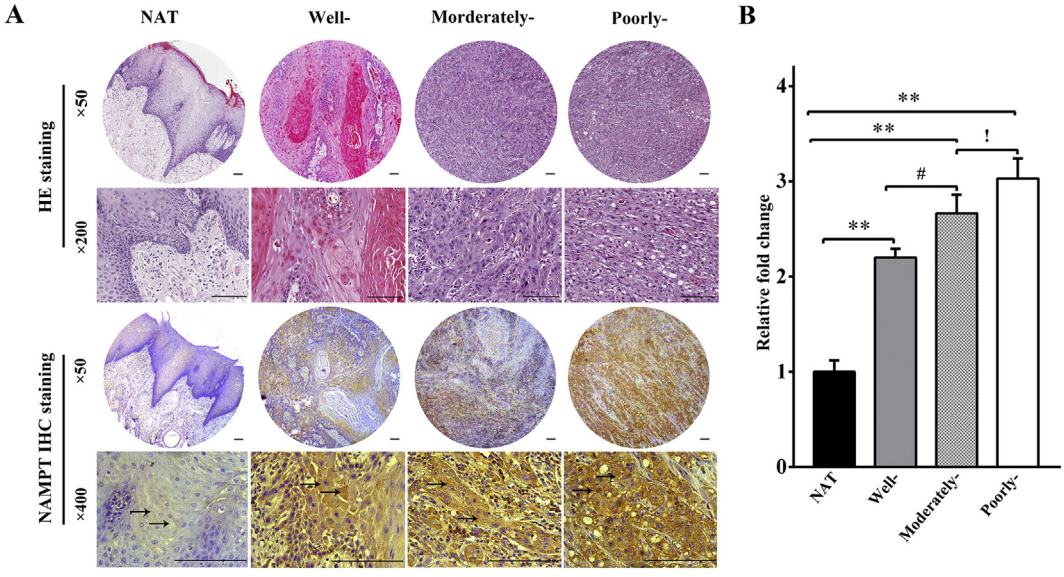

Fig. 1. Increased NAMPT expression was observed in OSCC and it was highly associated with the grades of differentiation. NAMPT expression was analyzed with tissue microarray (TMA). Positive immunostaining for NAMPT was brown and indicated by arrow. (A) Representative immunohistochemical staining of NAMPT expression. (B) Relative intensity fold change of NAMPT expression in normal and adjacent tissue (NAT) and OSCC tissues of three grades of differentiation. $^{**}p < 0.01$ *vs.* NAT; $^{\#}p < 0.05$ *vs.* well differentiated OSCC; $^{!}p < 0.05$ *vs.* moderately differentiated OSCC. Bar = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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