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Nuclear factor-kappa B inhibition can enhance therapeutic efficacy of ^{131}I on the in vivo management of differentiated thyroid cancer

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ABSTRACT

Aim: Nuclear factor-kappa B (NF-κB) plays a key role in cancer development and therapy resistance. We aimed to determine whether NF-κB inhibition can enhance ^{131}I efficacy in differentiated thyroid cancer (DTC) in vivo.

Main methods: Every nude mouse was ip injected with 1 mCi of ^{131}I for thyroid ablation. Four weeks later, DTC cells were implanted. Another six weeks later, mice received four types of therapies, namely control vehicle, 1 mCi of ^{131}I once, 10 mg/kg of Bay 11-7082 (a NF-κB inhibitor) trice and combination treatment. Pre-ablation ^{99m}Tc-pertechnetate imaging, post ablative and post therapeutic imaging were performed. Target-to-background ratios (T/Bs) on xenograft tumors were calculated and compared. Nuclear extract from tumor samples were assessed by DNA-binding assay and Western blot. Apoptotic indices by TUNEL assay were determined and tumor volume curve was drawn to compare therapeutic effects in different groups.

Key findings: Post therapeutic imaging displayed ^{131}I-avidity of xenograft tumors and completeness of thyroid ablation. T/Bs comparison showed no significant differences in mice received either ^{131}I mono-therapy or combined therapy. DNA-binding assay and Western blot showed enhanced function and expression of NF-κB by ^{131}I, which were inhibited substantially by Bay 11-7082 combination. Apoptotic indices were significantly increased by combined treatment than by any mono-therapy. And DTC lesional volumes were significantly regressed by combined treatment than by any mono-therapy.

Significance: We demonstrated that NF-κB inhibition can be a good interventional avenue to enhance therapeutic potentiation of ^{131}I on the in vivo management of DTC.

Introduction

Differentiated thyroid cancer (DTC) is the most frequent malignancy of the endocrine system. In the USA, its incidence is about 8.7 per 100,000 population in 2002 (Cooper et al., 2009). Nearly all patients with DTC should undergo total thyroidectomy. For the post-surgical management of DTC, radioiodine 131 (^{131}I) prevails as the cornerstone treatment strategy, which is depended on the retained ability of thyroid cancerous tissue to accumulate ^{131}I (Cooper et al., 2009; Gharib et al., 2010; Pacini et al., 2010). Nevertheless, mounting evidence indicates that there are some problems in the ^{131}I management of DTC. For instance, some metastatic DTC lesions have the tendency to diminish ^{131}I concentration capacity or to become ^{131}I-refractory (Durante et al., 2006; Pfister and Fagin, 2008). And for some cases, even ^{131}I-avid lesions could not be controlled by ^{131}I mono-therapy effectively (Als et al., 2002; Basaria and Salvatori, 2002; Miyauchi et al., 2008; Rosario et al., 2005; Takano et al., 2006; Tan et al., 2009).

During the past decade or so, serious efforts have been undertaken to address the above problems. Human sodium-iodine symporter (hNIS) transfection into hNIS-defective thyroid cancer cell line was demonstrated to restore the in vivo iodine accumulation (Haberkorn et al., 2004; Smit et al., 2002). Re-differentiation drugs like all-trans retinoic acid and histone deacetylase inhibitors could also increase the iodine uptake and retention in poorly differentiated and anaplastic thyroid cancer cell lines (Furuya et al., 2004; Malehmir et al., 2012). Another way to circumvent therapeutic resistance is to promote apoptosis. Nuclear factor-kappa B (NF-κB) has been demonstrated to play a crucial role in thyroid cancer development, chemotherapy resistance and radiotherapy resistance (Meng et al., 2008, 2012; Pacifico and Leonardi, 2010; Palona et al., 2006; Starenki et al., 2004a,b). Our recent in vitro experiment revealed that ^{131}I could induce NF-κB activation, which attenuated ^{131}I induced apoptosis. NF-κB inhibition was effective

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in suppressing NF-κB regulated anti-apoptotic changes and in combined regimen apoptosis was achieved synergistically (Meng et al., 2012). This work also showed the NF-κB inhibition approach did not significantly influence iodine uptake of DTC cells.

On the basis of the encouraging in vitro experiment, we aimed to determine whether the work could be translated to an in vivo setting. Thereby, the purpose of the present research was to test the hypothesis that NF-κB inhibition could enhance ^{131}I therapeutic efficacy in xenograft nude mice experiment.

Materials and methods

Cell culture

The human papillary thyroid carcinoma cell line KTC-1 was kindly provided by Dr. Shunichi Yamashita and Dr. Norisato Mitsutake (Research Centre for Genomic Instability and Carcinogenesis, and Radiation Medical Sciences Department of Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan). DTC cells were grown in Dulbecco's minimum essential medium (GIBCO BRL, NY, USA) supplemented with 5% fetal bovine serum (GIBCO BRL, NY, USA), 1% (w/v) penicillin/streptomycin (Sigma-Aldrich, MO, USA) and 1 mU/mL thyrotropin (Sigma-Aldrich, MO, USA) in a 5% CO_2 humidified atmosphere at 37 °C.

In vivo xenograft model protocol

Institutional review board of Tianjin Medical University approved the ethical and methodological aspects of the investigation. Three to four weeks old BALB/c nu/nu male mice were purchased from Laboratory Animal Center of Chinese Academy of Military Medical Sciences (Beijing, China). Each mouse received an intra-peritoneal (ip) injection with 1 mCi of ^{131}I (Beijing Atom HighTech, Beijing, China) for thyroid ablation. Two weeks after thyroid ablation, replacement levo-thyroxine (Merck Serono, Darmstadt, Germany) was provided in drinking water at a concentration of 12.5 μg/L. Four weeks after thyroid ablation, KTC-1 cells (5×10^6) were injected subcutaneously into one flank side. Then animals were randomly assigned into four groups (eighteen per group). At the end of the sixth week after tumor implantation, when tumor volume reached around 400 mm^3, treatments were ready to commence. Mice in group one received ip injection of saline, and served as control. Each mouse in group two was ip injected with 1 mCi of ^{131}I on day one of the seventh week after tumor implantation. On day one, two and three of the seventh week, each mouse in group three was ip injected with 10 mg/kg of Bay 11-7082 (Sigma-Aldrich, MO, USA). Mice in group four received combined therapy. During the sixth and seventh week, levo-thyroxine replacement therapy was stopped. The tumor xenograft sizes were measured with calipers on the last day of every week, and tumor volume was estimated using the formula tumor volume = length × width × height × 0.52 (Dwyer et al., 2006a,b; Hsieh et al., 2007; Spitzweg et al., 2000, 2001). For sixteen weeks in total, tumor volume changes were monitored. And body weight, feeding behavior and motor activity of each animal were used as indicators of general health.

In the study, Bay 11-7082 was used to inhibit NF-κB pathway. Bay 11-7082, with the full name of (E)-3-(4-methylphenylsulfonyl)-2-propenenenitrile, has a molecular weight of 207.25. It is provided with ≥98% purity (HPLC). It is an irreversible inhibitor of IκBα phosphorylation, which increases stabilization of IκBα and specifically blocks NF-κB signaling. Bay 11-7082 has been shown to be a potent inducer of apoptosis in a variety of cancer cell lines. Although Bay 11-7082 has not undergone clinical development, prior in vivo animal experiments showed its limited toxicity and good therapeutic effectiveness (Keller et al., 2006; Zheng et al., 2008).

In vivo imaging study

Before thyroid ablation, whole body imaging was performed 30 min after ip injection with 0.5 mCi of ^{99m}Tc-pertechnetate in each mouse. Images were obtained by using a dual-detector SPECT/CT equipped with low-energy collimators (Discovery VH; General Electric Medical Systems, Milwaukee Wisconsin, USA). Post ablative imaging and post therapeutic whole body imaging were performed four days after ^{131}I administration. Images were obtained by using the above SPECT/CT equipped with high-energy collimators. All images were formatted to a 256×256 matrix, and then processed by the eNTEGRA workstation (General Electric Medical Systems, Waukesha, Wisconsin, USA). In the post therapeutic images, a region of interest (ROI) surrounding the target xenograft lesion was drawn by using the method as described before (Dingli et al., 2003, 2004; Wang et al., 2010). Mean pixel counts in the target lesion was calculated. Then a circular background ROI with the size of 40 pixels was drawn on the upper thoracic area where the distribution of radiotracer was low and relatively homogeneous. Mean pixel counts in the background lesion was calculated as well. Target-to-background ratio (T/B) was defined as mean pixel counts in the target ROI over mean pixel counts in the background ROI (Wang et al., 2010). T/Bs in group one and group three were compared.

Preparation of nuclear extract from tumor samples

On day seven of the seventh week, six mice in each group were sacrificed to obtain xenograft tumor samples. Nuclear protein was extracted as described previously (Kunnumakkara et al., 2007; Rahman et al., 2006). Tumor tissues (75–100 mg) were minced and incubated on ice for 30 min in 0.5 mL of ice-cold buffer A [10 mmol/L HEPES (pH 7.9), 1.5 mmol/L KCl, 10 mmol/L $MgCl_2$, 0.5 mmol/L DTT, 0.5 mmol/L phenylmethylsulfonyl fluoride]. The minced tissue was homogenized using a Dounce homogenizer (Kontes Co., Vineland, NJ, USA) and centrifuged at 16,000 ×*g* at 4 °C for 10 min. The resulting nuclear pellet was suspended in 0.2 mL of buffer B [20 mmol/L HEPES (pH 7.9), 25% glycerol, 1.5 mmol/L $MgCl_2$, 420 mmol/L NaCl, 0.5 mmol/L DTT, 0.2 mmol/L EDTA, 0.5 mmol/L phenylmethylsulfonyl fluoride, 2 Ag/mL leupeptin] and incubated on ice for 2 h with intermittent mixing. The suspension was then centrifuged at 16,000 ×*g* at 4 °C for 30 min. The supernatant (nuclear extract) was collected and stored at −80 °C until use. Protein concentrations were determined with a bicinchoninic acid assay reagent kit (Sigma-Aldrich, MO, USA).

DNA-binding assay

The multi-well colorimetric assay for active NF-κB function was performed (Meng et al., 2008, 2012; Starenki et al., 2004a,b). Briefly, equal amounts of nuclear extracts were incubated in 96-well plates coated with immobilized oligonucleotide containing a NF-κB consensus binding site. NF-κB binding to the target oligonucleotide was detected with primary antibody specific to p65 subunit and HRP-conjugated secondary antibody. For quantification of activity, optical densities were measured at 450 nm with a Multiskan MS Plate Reader (Labsystems, Helsinki, Finland). Nuclear protein from each xenograft tumor sample was tested in quintuple.

Western blot

NF-κB expression was assessed by Western blot (Meng et al., 2008, 2012; Starenki et al., 2004a,b). Equal amounts of protein were electrophoresed by SDS-PAGE in 10% polyacrylamide gels, and then transferred onto nitrocellulose membrane (Amersham Biosciences, NJ, USA) by semidry blotting. Membranes were blocked with Tris-buffered saline/0.1% Tween 20 (TBST) containing 5% milk for 60 min at room temperature, and then immune-blotted with appropriately diluted primary antibodies at 4 °C overnight. After washing three times with TBST, the