Overexpression of IFN-γR Increases the Radiotherapy Resistance of Nasopharyngeal Carcinoma

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Abstract

Purpose: To assess the relationship between IFN-γR expression and clinical radiotherapy outcome in nasopharyngeal carcinoma.

Methods: IFN-γR expression was examined by immunohistochemistry from 70 nasopharyngeal carcinoma patients. Then, statistical analysis was conducted to explore the correlation between IFN-γR expression and tumor clinicopathological characteristics. Finally, CNE-1 cells were employed to detect the effects of IFN-γR expression on radiotherapy outcomes by in vitro assays.

Results: IFN-γR was upregulated dramatically in nasopharyngeal carcinoma tissues. Elevated expression of IFN-γR correlated significantly with tumor stage, positive lymph node metastasis and poor prognosis in patients with nasopharyngeal carcinoma. In vitro assays demonstrated that overexpression of IFN-γR promoted the radio-resistance ability in CNE-1 cells. Knockdown of IFN-γR facilitated CNE-1 cells more sensitive to radiation, and a wobble mutant of IFN-γR could restore this effect.

Conclusions: Overexpression of IFN-γR may correlate positively with radiotherapy resistance of nasopharyngeal carcinoma.

Keywords: IFN-γR; overexpression; prognosis; radiation resistance; NPC

Introduction

Nasopharyngeal carcinoma is one of the most common malignancies and occupies the seventh leading cause of cancer-related deaths in China [1]. Particularly in South China, nasopharyngeal carcinoma is the most prevalent. Locally, advanced nasopharyngeal carcinoma patients are generally treated with radiotherapy, and the prognosis is related with radiotherapy [2]. Both local-regional failure and early systemic dissemination of the disease always contribute to treatment failure, which makes radio-sensitization promising in improving the outcome of therapeutic irradiation [3].

IFN-γ plays a pleiotropic role in modulating immunity and antiviral activity. It stimulates macrophages and NK cells to produce MHC class I and II molecules, while IFN-γR is not required for the development of the immune system [4]. Researches showed that IFN-γ was overexpressed in breast cancer and its amplification was closely related with lymph node metastasis and poor prognosis [5-7]. Until now, there are still no reports about whether IFN-γR has an effect on the radiotherapy for NPC. In the present study, we found for the first time that IFN-γR expression correlated with the prognosis of NPC patients who received radiotherapy. High IFN-γR levels improved the radiation resistance ability of NPC cells.

Patients and Methods

Tissue samples

Seventy tissues of nasopharyngeal carcinoma were freshly collected by nasopharyngoscope and procured by the Department of Pathology, Cancer Center of Wuhan Union Hospital, China. The collection of tumor samples was approved by the institutional ethics committee. Informed consent forms had been signed for sample collection before. Tumor regions were technically separated by experienced pathologists and promptly stored at liquid nitrogen until needed. All patients included underwent concurrent chemotherapy (Cisplatin, 40mg/m², weekly) with radiation therapy for the first time. They received irradiation with daily fraction doses of 2.12Gy for a total dose 70 Gy, which was administered with linear accelerators. The clinicopathologic features of patients were showed in Table 1.

Immunohistochemical staining

Immunohistochemical staining was conducted as previously described. After rehydration, the paraffin-embedded nasopharyngeal carcinoma tissues were immersed in 3% hydrogen peroxide solution for 10 min, and then heated in EDTA buffer (pH 8.0) for 25 min. The slides were blocked with 10% normal rabbit serum at 37°C for 30 min, and then incubated with rabbit polyclonal antibody against primary antibody (IFN-γR, 1: 200, ABCAM) overnight at 37°C. The slides were washed with PBS, and then incubated with biotinylated second antibody (diluted 1: 200) for another 30 min at 37°C. Finally, streptavidin-peroxidase reactivity was detected with DAB solution. Cell nucleus was counterstained with hematoxylin. The immunohistochemical slides were graded according to the ratio of tumor cells with nuclear labeling. The level of IFN-γR- positive staining was scored into three grades: 0 for < 10%, positive for 10-30%, strong positive for > 30%.

Conclusion

Overexpression of IFN-γR increases the radiotherapy resistance of nasopharyngeal carcinoma. The upregulated IFN-γR has an effect on the clinical radiotherapy outcome in nasopharyngeal carcinoma patients.
Plasmid constructs and small interfering RNA synthesis

Double-stranded oligonucleotide with the following sequence was synthesized into the pSilencer 3.1- H1 neo small interfering RNA (siRNA) expression vector, and the scrambled siRNA was applied as control. Mutant DNA-IFN-γR (BOSTER, Wuhan, China) was used to resist the effect of IFN-γR-RNAi.

Cell culture and transfection

The CNE-1 cells (one of the esophageal squamous carcinoma cell lines) were cultured in RPMI 1640 (Invitrogen) with 10% fetal bovine serum. Cell transfection was carried out using Lipofectamine 2000 (Invitrogen) following the manufacturer’s instruction. Cells were harvested after transient transfection for 48 h. For stable transfection, cells were selected with 200 μg/ml G418, and passaged by serial dilution. During the next two weeks, colonies were screened by selective medium. The positive colonies with IFN-γR overexpression and IFN-γR knockdown were chosen for experiments.

Western blot analysis

Total proteins were isolated from patient tissues and cultured cells with lysis buffer [10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP40] containing protease inhibitors. After centrifugation (15,000 RCF, 30 min, 4°C), supernatants were recovered for immunoblot analysis. The proteins were subjected to SDS-PAGE and then transferred onto polyvinylidenedifluoride (PVDF) membranes (Millipore). Next, the membranes were blocked and then incubated with primary antibody against IFN-γR (1: 500, ABCAM) at 4°C overnight, then incubated with horseradish peroxidase-conjugated accordingly secondary antibody for 1 h at room temperature and developed using ECL detection reagent (BOSTER).

Ionizing radiation

CNE-1 cell line was exposed to IR in a JL Shepherd Model 143 137Cesium gamma and irradiated at a rate of 2.4 Gy/min.

Colonies were fixed in methanol and stained with Giemsa. Colonies were counted in each well. Each experiment was performed three times independently.

Results

**Overexpression of IFN-γR in esophageal squamous cell carcinoma**

IFN-γR expression was examined in 70 NPC samples and adjacent normal tissue by immunohistochemistry. Results showed that IFN-γR was hardly expressed or revealed weak expression in normal epithelial cells. While, in 36 cases of 70 NPC samples, IFN-γR expression exhibited significantly enhanced trend compared to the normal epithelium [Table 1]. Statistical analysis indicated that elevated expression of IFN-γR was significantly related to tumor stage, clinical stage and lymph node metastasis (p < 0.05) [Table 2].

**Table 2.** Correlation between IFN-γR overexpression and tumor clinicopathologic features.

<table>
<thead>
<tr>
<th>Clinicopathologic features</th>
<th>Number of Cases</th>
<th>Negative</th>
<th>Positive</th>
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<tr>
<td>Age</td>
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<tr>
<td>T4</td>
<td>14</td>
<td>7</td>
<td>7</td>
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<td>Lymph node metastasis</td>
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<td>2</td>
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<td>28</td>
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Next, the effect of IFN-γR expression on patient’s survival was analyzed. IFN-γR expression is positive in some patients and negative positive in others [Figure 1A]. After the included patients underwent chemotherapy combined with radiation therapy (55 out of 70), compared to NPC patients with low IFN-γR expression in cancerous tissue was significantly higher, patients with cancerous tissue of high IFN-γR expression showed significantly lower survival rate (p = 0.001) [Figure 1B].
Effects of IFN-γR on the radio-sensitivity of CNE-1 cells

To confirm the clinical data, CNE-1 cells were radiated with a dose of 5 Gy for 0, 2, 4, 8 and 12 h to know IFN-γR expression change. IFN-γR expression was increased after radiation in a time-dependent manner, which implied that IFN-γR may be associated with the radiation response of CNE-1 cells [Figure 2].

Colony formation assay was conducted to detect the effects of IFN-γR on the radio-sensitivity of CNE-1 cells [8]. IFN-γR knockdown cells showed a significantly lower survival fraction than control groups [Figures 3A and B]. Conversely, the survival fraction of cells with forced IFN-γR expression was higher [Figures 3C and D]. Therefore, results indicated a positive relationship between IFN-γR expression and radio-sensitivity of CNE-1 cells.

Discussion

Overexpression of IFN-γR has been observed in various types of human tumors, and elevated expression of IFN-γR indicated poor prognoses for patients, such as gastric carcinoma, prostate cancer, oral squamous cell carcinoma and non-small cell lung cancer. Particularly, IFN-γR hyperexpression was found to be associated with poor prognosis and reduced p27 expression in gastric carcinoma. Some studies have revealed that IFN-γR was amplified and upregulated in breast cancer. Moreover, its overexpression indicated worse tumor stage and lymphatic metastasis. However, there have been no reports on IFN-γR expression in NPC until now. Our results showed for the first time that IFN-γR expression increased in NPC, which significantly related with tumor stage and positive lymphatic metastasis. Coincidently, other studies have demonstrated that elevated IFN-γR level was correlated with poor prognosis in colorectal carcinoma [9,10]. However, no significance was shown between IFN-γR level and overall survival of NPC patients in the present study. We could not help putting forward the speculation whether IFN-γR expression affects radiotherapy outcome. Survival analysis unveiled that the overall survival rate of patients received radiation therapy was indeed affected by IFN-γR expression. Histological detection also showed a strong association between IFN-γR hyperexpression and T-stage and lymphatic metastasis. Besides, in vitro assay further confirmed this point. This was the first report about the correlation between IFN-γR expression and radiation resistant of carcinoma.

Some studies reported that downregulation of IFN-γR suppressed prostate cancer cell proliferation, anchorage-independent growth and migration [11,12], while knockdown of Cks2 induced apoptosis [13,14]. Decreased IFN-γR promoted G2/M phase arrest and programmed cell death in human lung cancer cells [15]. Some study also found that overexpression of IFN-γR in breast cancer cells repressed cell apoptosis through the MEK-Erk pathway [16]. However, no reports have described the apoptotic effects of IFN-γR expression in radiation-treated cells. In our next study, we may find out that overexpression of IFN-γR could induce apoptosis in CNE-1 cells after radiation.

Figure 1. Representative IFN-γR immunostaining in NPC and effects of IFN-γR expression on patient’s survival. A: IFN-γR expression is positive in some patients and negative positive in others. B: In the patients that received concurrent chemotherapy with radiation therapy (55 out of 70), the survival rate of patients with low IFN-γR expression was significantly higher than that of patients with high IFN-γR expression (p = 0.001).
Gang Peng (2017) Overexpression of IFN-γR Increases the Radiotherapy Resistance of Nasopharyngeal Carcinoma

To conclude, this study identified that overexpression of IFN-γR correlated with the poor prognosis of NPC patients receiving radiotherapy and elevated IFN-γR expression promoted the radiation resistance ability of NPC cells.

Conflict of interest: None declared.

Source of funding: None.

Consent: Written informed consent was obtained from the patients.

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References


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Citation: