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MicroRNA-196b enhances the radiosensitivity of SNU-638 gastric cancer cells by targeting RAD23B

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ABSTRACT

Gastric cancer is characterized by resistance to ionizing radiation. The development of resistance to radiotherapy in gastric cancer patients is one of the obstacles to effective radiotherapy. MicroRNAs are small well-conserved non-coding RNA species that regulate post-transcriptional activation. Our study aimed to investigate the role of miR-196b in radiation-induced gastric cancer. In the present study, we found that miR-196b expression was significantly reduced following radiation. The ectopic miR-196b expression sensitized SNU-638 gastric cancer cells and increased γ-H2AX foci upon radiation treatment. Bioinformatics analysis suggested that the DNA repair protein RAD23B was a putative target gene of miR-196b. Overexpression of miR-196b suppressed RAD23B expression in SNU-638 cells. Reporter assays further showed that miR-196b inhibited RAD23B 3′-UTR luciferase activity. Knockdown of RAD23B by small interfering RNA transfection closely mimicked the outcomes of miR-196b transfection, leading to impaired DNA damage repair in gastric cancer cells. Our results show that miR-196b improved radiosensitivity of SNU-638 cells by targeting RAD23B. Our data indicate that miR-196b is a potential target to enhance the effect of radiation treatment on gastric cancer cells. These findings will provide evidence for a new therapeutic target in radiotherapy.

1. Introduction

MicroRNAs (miRNAs) are small non-protein-coding single-stranded RNAs that regulate post-transcriptional gene expression by either inhibiting mRNA translation or inducing mRNA degradation. miRNAs participate in a wide variety of physiological and pathological cellular processes, such as cell differentiation, proliferation, and apoptosis [1,2]. Given this wide range of functions, several miRNAs have emerged as candidate oncogenes and tumor suppressors involved in the networks specifically altered during tumorigenesis or metastasis [3–5]. The expression of miR-196b is elevated in gastric cancer tissues compared to normal tissue [6,7]. Elevated circulating miR-196b level is highly correlated with metastatic potential and poor survival [8]. Therefore, miRNAs may be useful as biomarkers to identify and classify various tumors.

Ionizing radiation (IR) is an important treatment modality used for various malignancies, and it induces oxidative genotoxic stress. Ionizing radiation causes severe cellular damage and stress, both directly via energetic disruption of DNA integrity and indirectly as a result of intracellular free radical formation [9]. YAP1, a effector of Hippo signaling is reported to be negatively regulated by tumor suppressor miRNAs including miR-15a, miR-16-1 and miR-506 in gastric cancer [10–12]. In a previous report, miR-375 desensitizes cells to ionizing radiation and etoposide, and miR-375 abrogates the cell cycle arrest and apoptosis after DNA damage [13]. Gastric cancers are commonly treated with chemoradiotherapy or adjuvant treatment with surgery. Since gastric cancers have low radiosensitivity, no survival differences have been observed in gastric carcinoma patients treated with radiation therapy [14–16]. Thus, the development of resistance to radiotherapy in gastric cancer patients is an obstacle for effective radiotherapy.

In this study, we hypothesized that alterations in miRNA expression may improve the radiation response of gastric cancer cells. Our data indicate that miR-196b, a downregulated miRNA after IR treatment, plays an important role in the radiosensitivity of human cancer cells by targeting RAD23B.

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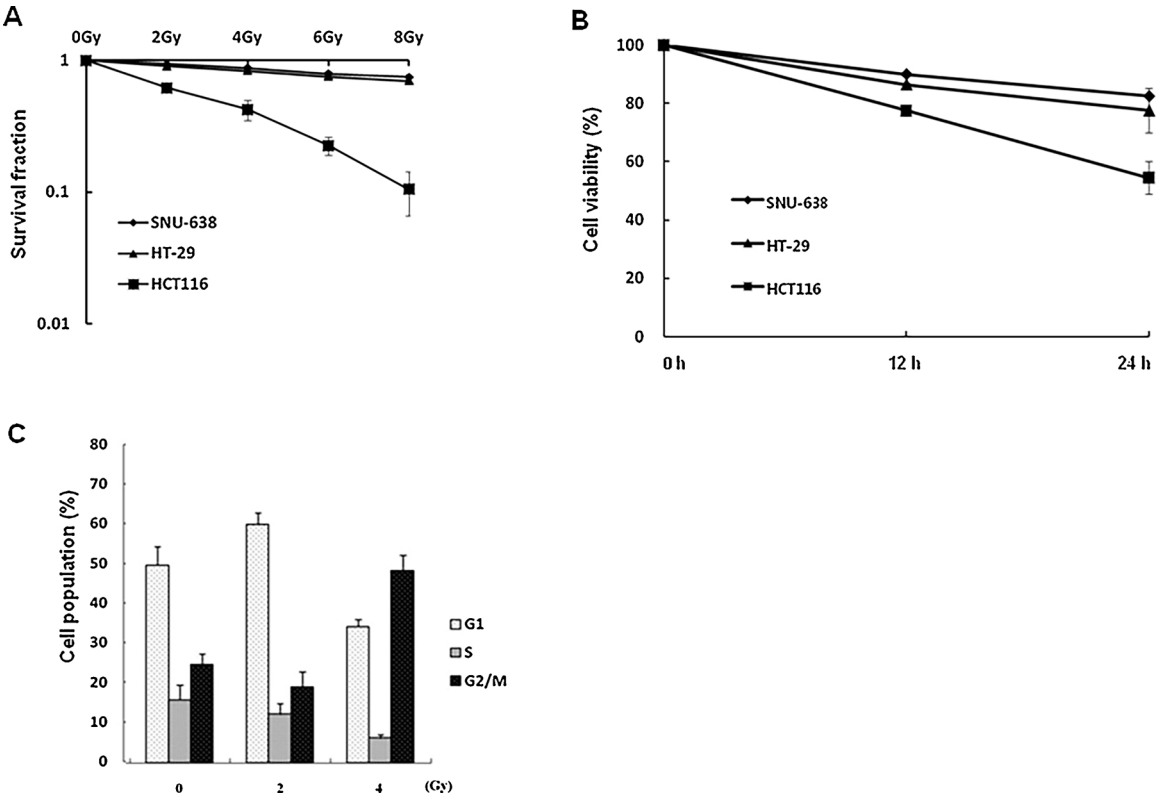

Fig. 1. The expression of miR-196b in SNU-638 gastrointestinal carcinoma cells exposed to IR. (A) Survival in response to different doses of IR was evaluated in gastrointestinal cancer cells via the clonogenic assay. (B) Time-dependent cell viability of gastrointestinal cancer cells in response to 4 Gy IR was determined by trypan blue assay. Error bars indicate SE of the mean of duplicate measurements from three independent experiments. (C) Cell cycle analysis of irradiated human SNU-638 cells. Cells were exposed to IR and incubated at 37 °C for 24 h. The DNA contents of SNU-638 cells in the G1, S, and G2/M phases of the cell cycle are shown.

2. Materials and methods

2.1. Cell culture and irradiation

SNU-638, HCT116, and HT-29 were cultured at 37 °C in RPMI-1640 or MEM containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin in a humidified chamber supplemented with 5% CO_2. For irradiation, 1×10^6 cells were seeded in 100-mm culture dishes. At 24 h post-seeding, cells were exposed to 2, 4, 6, and 8 Gy with a Gammacell® 3000 Elan Irradiator (^{137}Cs A-ray source; MDS Nordin, ON, Canada). At specific time points, the medium was removed, and cells were washed and harvested.

2.2. Clonogenic assay

Cells were plated in six-well plates and then irradiated with the appropriate radiation dose. Cells were incubated until colonies containing at least 50 cells were formed. After incubation for 10 days, cells were fixed with 70% ethanol and stained with 0.5% crystal violet. The surviving fraction was calculated as the following: (the number of colonies formed)/(the total number of cells plated × the plating efficiency). The surviving fraction was further plotted in log scale.

2.3. Cell viability assay

Cells were detached by trypsinization, and the cell suspensions were stained with 0.4% trypan blue solution (Sigma-Aldrich, St. Louis, MO, USA). The percentage of viable cells was quantified by counting a minimum of 200 cells per cell passage. Three independent experiments were performed in duplicate.

2.4. Cell cycle analysis

Cells were cultured to 70–80% confluence and exposed to radiation (2 Gy or 4 Gy). At 24 h after radiation treatment, cells were fixed in ice-cold 70% ethanol. Cell pellets were washed with PBS and then suspended in 40 μg/mL propidium iodide containing 50 μg/mL RNAse, and analyzed for cellular DNA content using a FACSCalibur flow cytometer (BD Biosciences, Franklin, NJ, USA). CellQuest Pro software was used to determine the percentages of cells in the G1, S, and G2/M phases of the cell cycle.

2.5. Gene expression analysis by microarray

Total RNA was isolated from irradiated cells using TRIzol reagent (Invitrogen, Eugene, OR, USA). For biotin labeling to 5′-end of uncapped RNA, each 5 ~ 10 μg of total RNA were labeled with Enzyme L, followed by incubation for 3 h at 37 °C. The biotin-labeled RNA were combined with the same volume of Hyb buffer (GenoSensor, USA), boiled for 5 min, and pipetted onto GenoExplorer™ miRNA Biochip (GenoSensor, Tempe, Arizona, USA). Hybridization was performed for 16 h at 42 °C using LifterSlip™ (GenoSensor, Tempe, Arizona, USA). After hybridization incubation, first wash was performed and microarrays were spin-dried. For fluorescent dye staining, SA-S (streptavidin-stain) dye was added onto the chips on which the biotin-labeled RNAs were hybridized, followed by incubation for 30 min at 25 °C. Finally, hybridized microarrays were washed to remove non-specific binding, dried, and scanned with GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA, USA). The hybridized images were quantified with GenePix Software (Molecular Devices, Sunnyvale, CA, USA). All data normalization and selection of fold-changed genes were performed using GeneSpringGX 7.3 (Agilent Technology, Santa Clara, CA, USA). The averages of normalized ratios were calculated by dividing the