Early Changes in Apparent Diffusion Coefficients Predict Radiosensitivity of Human Nasopharyngeal Carcinoma Xenografts

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Objectives/Hypothesis: Our objective was to predict the radiosensitivity of human nasopharyngeal carcinoma xenografts in nude mice models through an examination of early changes in apparent diffusion coefficient (ADC) values.

Study Design: Randomized.

Methods: BALB/c-ncu nude mice (n = 20) were divided into two groups that were subcutaneously injected with CNE1 or CNE2 cell lines. Xenograft volumes were measured after tumor formation, mice were scanned with a diffusion-weighted imaging sequence, and the mean ADC values were measured (ADC0). Fifteen to 20 hours after tumors received 15 Gy, mice were scanned again and ADC values (ADC1) were measured.

Results: ADC0 and ADC1 values of the CNE1 group showed no significant difference (P = .692). The difference between the ADC0 and ADC1 values of the CNE2 group was statistically significant (P < .001). ADC0 values of the two groups exhibited no statistically significant difference (P = .204). ADC1, ADC1-0, and ΔADC of the two groups exhibited statistically significant differences (P < .001; P = .001 and .002, respectively). After irradiation, volume changes ΔV8, ΔV10, and ΔV12 of two groups were statistically different (all P < .001). Pearson correlation analysis showed ADC1-0 and ΔADC were positively correlated with ΔV8, ΔV10, and ΔV12. The cut point was found by means of a receiver operating characteristic curve, and the ΔV12 of the two redivided groups showed a statistically significant difference (P = .001).

Conclusions: This study found that changes in ADC values correlated with volume changes after irradiation. Therefore, ADC values have the potential to predict the radiosensitivity of nasopharyngeal carcinoma xenografts.

Key Words: Nasopharyngeal carcinoma, nude mice, magnetic resonance imaging, diffusion weighted imaging, apparent diffusion coefficient, radiosensitivity.


INTRODUCTION

Nasopharyngeal carcinoma is one of the most common cancers found in East Asia, particularly southeast China, and Africa. Because of its biologic behavior and anatomic characteristics, the most common treatment for nasopharyngeal cancer is radiotherapy or comprehensive chemotherapy administered before radiotherapy. Technologic advances in radiation therapy, especially the introduction of intensity-modulated radiotherapy (IMRT), have improved dose distribution while sparing surrounding normal tissues, thereby greatly increasing the curative effect of radiotherapy for nasopharyngeal carcinoma. At present, the 5-year overall survival rate for patients treated with IMRT is approximately 83%, and the 5-year local control rate is approximately 93%; however, local recurrences and distant metastases are still the predominant causes of failure. Nearly 95% of nasopharyngeal neoplasms are poorly differentiated or undifferentiated squamous cell carcinoma. Outcomes vary extensively, with some patients who receive 40 Gy experiencing long-term control, and others who receive more than 80 Gy experiencing residual tumors in the nasopharynx. Moreover, patients in the same clinical stage can have very different prognoses that are related to tumor-specific biologic characteristics, including radiosensitivity. The ability to predict a particular tumor’s radiosensitivity before or during early stages of treatment has clinical significance for individualized treatment plans and prescription doses. Tumor radiosensitivity is determined by cancer tissue type, tumor microenvironment, and whole body condition; the definitive factor is a tumor’s inner radiosensitivity, to which some factors are closely related, such as apoptosis, proliferation, vasculogenesis, and DNA and/or chromosomal damage. Although previous studies have found biomarkers and signaling pathways that are associated with radiosensitivity, these findings are still inapplicable to actual clinical practice. Currently, no effective method to predict the radiosensitivity of nasopharyngeal carcinoma exists.
Diffusion-weighted imaging (DWI) is a method of magnetic resonance imaging (MRI) that utilizes the movement of water molecules to produce images that indirectly reflect information regarding cell density and microstructures in living tissues. As far as we know, radiotherapy can induce apoptosis. In the early stage of this process, cells shrink, the extracellular space increases, and the diffusion movement of water molecules changes; thereafter, changes can be quantitatively described by the apparent dispersion coefficient (ADC). DWI can detect changes in tumor size and shape before they are visible to the naked eye. A number of studies have confirmed that early ADC values can be used as a sensitive multicancer biomarker to predict treatment responses,\textsuperscript{10,11} and some have found that ADC values can be used for the diagnosis of nasopharyngeal carcinoma\textsuperscript{12} and prediction of curative effect for radiotherapy and prognosis,\textsuperscript{13} but the utility of DWI to predict the radiosensitivity of nasopharyngeal carcinoma has not been investigated. We hypothesized that early changes in ADC values after irradiation could forecast the radiosensitivity of human nasopharyngeal carcinoma xenografts in nude mice. Using CNE1 and CNE2 cell lines with different levels of radiosensitivity, we established animal models and then applied DWI to achieve ADC values and test this hypothesis.

**MATERIALS AND METHODS**

All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in Fujian Medical University, Fuzhou, China, and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

**Human Nasopharyngeal Carcinoma Xenograft Models**

Twenty 5-week-old, male, BALB/c-nu nude mice, weighing 20 ± 2 g, were raised in the specific pathogen-free environment of the Laboratory Animal Center of Fujian Medical University (License No.: SYXX [Fujian] 2008-0001, Fuzhou, Fujian Province, China). Mice were divided into groups of 10 and subcutaneously injected with 0.1-Ml cell suspension (concentration 1 × 10\textsuperscript{7}/mL) of either highly differentiated human nasopharyngeal squamous cell carcinoma cell line CNE2 or poorly differentiated nasopharyngeal squamous cell carcinoma cell line CNE1 (Radiation Biological Laboratory, Fujian Tumor Hospital, Fuzhou, Fujian Province, China).

**Irradiation**

Mice were anesthetized with 5% chloral hydrate and fixed in a custom restraint. Xenografts were covered with gauze coated in 1 cm of petroleum jelly and received a single fraction of 15 Gy at a dose rate of 500 U/minute with a field size of 4 × 4 cm via a 6-MV x-ray generated by a medical linear accelerator (D6000CD Varian Medical Systems, Inc., Palo Alto, CA).

**MRI and Analyses**

MRI was performed before and 15 to 20 hours after irradiation. Mice were anesthetized with 5% chloral hydrate, restrained in a prone position, and fixed in alginate stamping materials (20 g alginate stamping powder mixed with 60 mL water at room temperature) at a height of 1.5 cm.\textsuperscript{14} Two to 3 minutes after the material solidified, mice were scanned with a 1.5-T MRI system using 3-inch round coils (gradient strength = 40 mT/m, slew rate = 150 mT/(M.S)); Excite III Signa Echo-Speed HD, GE Healthcare, Waukesha, WI, USA). The imaging protocol consisted of the following sequences:

Conventional axial MRI images were obtained using a fast spin-echo T2-weighted proton density (PD)–weighted image sequence (3,400/30 ms repetition time [TR]/echo time [TE] with a 2-mm slice thickness, a 0.6-mm interslice gap, a 10 × 8-mm field of view [FOV], a 256 × 224 acquisition matrix, and 2 excitations).

Axial diffusion-weighted images were obtained using a spin echo–echo planar imaging sequence (5,000/minimum ms TR/TE with a 2-mm slice thickness, a 0.6-mm interslice gap, a 10 × 10-mm FOV, and a 96 × 96 acquisition matrix) with a chemical-shift selective fat-suppression technique. The sequence was repeated for two values of motion-probing gradients (B = 0 and 600 s/mm\textsuperscript{2}).

Images were transferred to work station ADW 4.2 (GE Healthcare, Waukesha, WI, USA) and evaluated by two experienced radiologists. The ADC maps were then reconstituted for each pixel with B factors of 0 and 600 s/mm\textsuperscript{2} using the standard software (function 2) on the station. To measure the mean ADC values for each tumor, we chose the image with biggest xenograft volume, on which the xenograft could be clearly defined. Then we manually drew the outline of the xenografts on the ADC maps, and these were defined as regions of interest (ROIs). Each ROI was manually drawn to include as much of the tumor as possible. The mean ADC measurement was performed separately by two readers and the measuring results were averaged.

ADC values before and after irradiation were defined as ADC\textsubscript{0} and ADC\textsubscript{1}, respectively. The difference in value was defined as ADC\textsubscript{1-0}, change in ADC values before and after irradiation was defined as ΔADC, and ΔADC = ADC\textsubscript{1-0}/ADC\textsubscript{0} × 100%.

**Xenograft Volume**

A caliper (Tricle Brand, 0–150 mm; Shanghai Huiyi Measuring Instruments Co., Ltd., Shanghai, China) was used to measure the long (horizontal) diameter and the short (vertical) diameter of xenografts every other day. The volumes of the xenografts were calculated by \( V = ab^2/2 \), where \( V \) is volume of the xenograft \( x \) days after irradiation; \( a \) is the long diameter of the xenograft; and \( b \) is the short diameter of the xenograft. The change in xenograft volume \( x \) days after irradiation was defined as \( ΔV \) and \( ΔV = (V_x - V_0)/V_0 \).

**Statistical Analysis**

Statistical analysis was performed with SPSS 16.0 software packages (IBM Corporation, Armonk, NY). Normality tests of quantitative data were performed with a two-tailed one-sample Kolomogorov-Simirnov test; \( P < .1 \) indicated a statistically significant difference. The difference between groups was tested by independent \( t \) tests. In cases where variance between groups was unequal, we added a correction to compensate for unequal variance. For comparison of the differences inside one group, we instead used the paired \( t \) test. The relationship between \( ΔV \) and ADC values was analyzed using Pearson correlation analysis. Mice were first divided into groups based on \( ΔV_{12} \) being positive or negative, and we then used receiver operating characteristic (ROC) curves to acquire an optimal cut
value. Thereafter, mice were redivided into two new groups based on cut point, and the difference of $D_{V12}$ between both groups was compared. Two-tailed $P < .05$ values were considered statistically significant.

A mouse from the CNE1 group expired due to an anesthesia accident; thus, numbers entered for statistical analysis were nine for CNE1 and 10 for CNE2. All data involved in this study obeyed normal distribution ($P > .1$).

**RESULTS**

**Changes of ADC values**

The ADC values were compared before and after irradiation. No statistically significant differences between ADC_0 and ADC_1 for the CNE1 group were found ($t = -0.411, P = .692$). As Figure 1 illustrates, the CNE2 group exhibited a significantly lower ADC_1 than ADC_0 ($t = 6.606, *P < .001$). Table I shows that the differences between ADC_1, ADC_1-0, and $\Delta$ADC for the CNE1 and CNE2 groups were all statistically significant ($P < .001, P = .001$ and .002, respectively).

<table>
<thead>
<tr>
<th>ADC</th>
<th>CNE1</th>
<th>CNE2</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADC_0 ($\times 10^{-4}$mm$^2$/s)</td>
<td>18.8 ± 4.52</td>
<td>16.47 ± 3.12</td>
<td>.204</td>
</tr>
<tr>
<td>ADC_1 ($\times 10^{-4}$mm$^2$/s)</td>
<td>19.6 ± 4.55</td>
<td>8.47 ± 3.06</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>ADC_1-0 ($\times 10^{-4}$mm$^2$/s)</td>
<td>0.8 ± 5.84</td>
<td>-7.99 ± 3.83</td>
<td>.001</td>
</tr>
<tr>
<td>$\Delta$ADC (%)</td>
<td>10.22 ± 44.33</td>
<td>-46.74 ± 22.82</td>
<td>.002</td>
</tr>
</tbody>
</table>

$\text{ADC} =$ apparent diffusion coefficient.

**DISCUSSION**

At present, an early, noninvasive, and clinically applicable method that can predict the radiosensitivity of nasopharyngeal neoplasms does not exist. Our study found that differences in ADC values for nude mice bearing human nasopharyngeal carcinoma xenografts before and 15 to 20 hours after irradiation had a positive correlation with xenograft-volume changes at 8, 10, and 12 days after irradiation. Mice were divided into two groups based on $\Delta V_{12}$ being positive or negative. The cut point of ADC_1-0 was determined to be $\frac{-3.55 \times 10^{-4}}{\text{mm}^2/\text{s}}$ (sensitivity was 0.875, specificity was 0.909, and area under the curve was 0.932) by means of an ROC curve (Fig. 3). Nineteen mice were redivided into two groups based on ADC_1-0 $\geq \frac{1}{\text{2}}$20 of $\frac{-3.55 \times 10^{-4}}{\text{mm}^2/\text{s}}$, and then $\Delta V_{12}$ were 44.46 ± 42.00% and 37.13 ± 21.27% for the two groups, respectively. A significant statistical difference was found between the $\Delta V_{12}$ for the two groups ($t = 5.045, P = .001$).
We also found that xenograft volumes continued to have value in prediction of tumor sensitivity to radiation. This is confirmed by an ROC curve, indicating that changes in ADC values have the potential to predict the radiosensitivity of nasopharyngeal carcinoma xenografts.

MRI DWI is very sensitive to the diffusion of molecules in living tissues. Extracellular fibers, cell organelles, and biologic macromolecules affect the diffusion of water molecules. Whole-tissue diffusion is composed of extracellular water-molecule diffusion, transmembrane diffusion, and intracellular diffusion. Every part of water-molecule diffusion contributes differently to the whole-tissue diffusion. Therefore, difference or alteration of microstructures at the cell level will change the whole diffusion coefficient.17 Nude mice bearing human nasopharyngeal carcinoma xenografts will take place inflammatory reactions at an early stage after irradiation, which include the increased expressing of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), increased permeability of blood capillary, interstitial space edema, and interstitial pressure rise. All of these phenomena lead to vascular compression, decreased perfusion of tumor tissue microcirculation, which can cause the decrease of water molecule’s diffusion ability, and decline in ADC values; at the same time, increased expression of VEGF and FGF can also lead to increase of cell membrane permeability, swelling of cell and organelles, and decrease of intercellular space and can then limit the diffusion ability of water molecules and further exacerbate the decline of ADC values. On the other hand, after irradiation, increase of cell membrane permeability can make water molecules flow in and out of cell membranes much easier, which causes ADC values to rise. In addition, at an early stage after irradiation, some tumor cells may experience necrosis or apoptosis and then break into pieces, and this causes cell density to decrease, thus causing the diffusion ability of water molecules to increase and ADC values to rise. But at the early stage of irradiation, cells and stoma edema have a large proportion, so ADC values represent a decrease at this time. CNE1 and CNE2 in our study were from the nasopharynx epithelial cell line of nasopharyngeal neoplasm patients with highly differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma, and they are generally used in studies of nasopharyngeal neoplasm. CNE2 is much more sensitive than CNE1. Our study found that ADC values of the CNE2 group significantly decreased compared with those of the CNE1 group, suggesting that the noninvasive monitoring of ADC might have value in prediction of tumor sensitivity to radiation. We also found that xenograft volumes continued to increase 15 to 20 hours after irradiation; however, at this time point, we could not distinguish a difference in xenograft volume between the two groups. But at the same time, ADC values for the CNE2 group decreased significantly after irradiation, while the CNE1 group exhibited no significant difference. These results show that ADC values of xenografts with different levels of radiosensitivity changed dramatically, and changes of ADC values can reflect the difference of radiosensitivity compared with volume changes 15 to 20 hours after irradiation. Because ionizing radiation kills tumor cells in a log-cell kill manner, one fraction of radiation cannot kill all tumor cells. As the complete removal of dead cells is not immediate and some cells proliferate several times before they lose mitotic abilities, xenograft volumes continue to increase 15 to 20 hours after irradiation. But changes of ADC values stand for water diffusion changes at the micro level, so changes of ADC values are seen much earlier than volume changes.

Larocque et al.18 tested nude mice bearing human gliomas and found that changes in ADC values for less radiosensitive tumors were smaller than those found in more radiosensitive tumors. Dudeck et al.19 treated hepatic metastasis of colorectal cancer with yttrium-90 (90Y) microspheres, evaluated the treatment efficiency 6 weeks later, and found that ADC values of the reaction group (nodule volume decreased) decreased significantly, while ADC values of the nonreactive group (nodule volume did not decrease) increased. By means of an orthotopic animal model bearing human head and neck squamous cell carcinoma xenografts, Hamstra et al. found that 3 days after irradiation, ADC values decreased20, these findings are similar to our own.

Many studies examining ADC values to predict the therapeutic effect of radiotherapy have concluded that rising ADC values indicate a better therapeutic effect.

| Table III. Correlation Analysis of ∆Vx and Apparent Diffusion Coefficient Values. |
|-------------------------------------|----------------|----------------|----------------|
| ∆Vx                               | ADC0 r P Value | ADC1-0 r P Value | ∆ADC r P Value |
| ΔV9                               | 0.001 .998     | 0.623 .004       | 0.561 .013     |
| ΔV10                               | 0.163 .504     | 0.604 .006       | 0.513 .025     |
| ΔV12                               | 0.227 .349     | 0.641 .003       | 0.568 .011     |

ADC = apparent diffusion coefficient; ∆Vx = volume changes after irradiation.
these results do not parallel our findings.21–24 Notably, these studies used assessment times from days to months. After longer time periods after treatment, tumor cells become apoptotic or necrotic, cell density decreases, extracellular space increases, and the restriction of water-molecule diffusion loosens. Finally, ADC values rise. Hamstra et al. and his colleagues found that ADC values after irradiation showed a decreasing trend the third day, followed by an increasing trend after that time.20 Our research selected time points as early as 15 to 20 hours after irradiation, which was earlier than all the previously mentioned studies; consequently, we found that ADC values for the highly radiosensitive CNE2 group were much lower after irradiation, indicating that tumor radiosensitivity may be predicted at early time points after irradiation.

Some research shows that due to hypoxia or other factors, radiosensitivity inside a tumor is inhomogeneous. Using animal models bearing human glioma xenografts, Moffat et al.25 found that changes in ADC values inside a tumor were not homogeneous. Although our study only discussed the changes of whole-tumor mean ADC values before and after irradiation and did not probe the spatial distribution of water-molecule diffusion within a tumor, our research is, to our knowledge, still the first to report the ability of ADC values to predict the radiosensitivity of nasopharyngeal carcinoma. After further study of the underlying mechanism of this phenomenon, we hope to test MRI DWI in clinical practice to examine diffusion changes of tumors in humans during early stages of radiotherapy. If this method accurately predicts nasopharyngeal carcinoma radiosensitivity, it will make tailored therapies a reality. Treatment doses should be adjusted for patients with poor radiosensitivity, whereas doses should be lowered for patients with high radiosensitivity to reduce complications without reducing curative effects.

CONCLUSION

Our study applied MRI DWI to obtain ADC values of nasopharyngeal carcinoma xenografts with different levels of radiosensitivity. Changes in these values were dependent upon neoplasm radiosensitivity and correlated with volume changes after irradiation. Therefore, ADC values have the potential ability to forecast the radiosensitivity of nasopharyngeal carcinoma xenografts.

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BIBLIOGRAPHY


