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ORIGINAL RESEARCH–HEAD AND NECK SURGERY

Correlation of biomarkers in head and neck squamous cell carcinoma

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ABSTRACT

OBJECTIVE: To evaluate the relationship of functional magnetic resonance imaging (MRI) parameters, including choline/creatine ratio (Cho/Cr) and apparent diffusion coefficient (ADC) with protein expression of 10 common tumor and prognostic markers in head and neck squamous cell carcinoma.

STUDY DESIGN: Cross-sectional study.

SETTING: University hospital.

SUBJECTS AND METHODS: The Cho/Cr and ADC obtained from 74 patients with head and neck squamous cell carcinoma were correlated with the expression level of the 10 protein markers as determined by immunohistochemistry.

RESULTS: Cho/Cr showed significant positive correlations with cyclooxygenase 2 in primary tumors ($r = 0.714$), and epidermal growth factor receptor in metastatic cervical lymph nodes ($r = 0.522$). ADC showed significant ($r = -0.591$) negative correlation with human epidermal growth factor receptor 2 in metastatic cervical lymph nodes.

CONCLUSION: There are relationships between protein and functional MRI markers. Future research in this direction may improve our understanding of the cancer micro-environment.

The evaluation of head and neck carcinomas, most of which are histologically squamous cell carcinoma (head and neck squamous cell carcinoma [HNSCC]), frequently relies on imaging findings because the head and neck site possesses complex anatomy. Hence, radiological assessment plays a crucial part in the diagnosis and preoperative assessment of HNSCC. Magnetic resonance spectroscopy (MRS) provides a noninvasive modality to supplement magnetic resonance imaging (MRI) examination by evaluating the metabolic milieu of the tumor.[1] In vivo proton (1H) MRS is the most commonly used MRS method in clinical settings. Typically, the MRS assessment of tumors shows elevated metabolites of choline compounds (free choline, phosphocholine, and glycerophosphocholine), detected at 3.2 ppm, and this elevation is usually quantified as choline/creatine ratio (Cho/Cr) (as the level of creatine is usually constant).

Diffusion-weighted imaging (DWI) with magnetic resonance imaging (MRI) is a noninvasive technique that has been shown to be promising in the evaluation of primary malignancy in the head and neck region.[2] This technique measures differences in the relative diffusion of water protons between tissues. Normally, water protons exhibit random motion (Brownian movement). On diffusion-sensitive sequences, this motion causes phase dispersion of the MR signals and results in signal loss that can be quantified by the apparent diffusion coefficient (ADC), a reflection of the specific diffusion capacity of protons in a specific biological tissue.[3,4] Generally, any cellular or interstitial structures that hinder water proton movement, such as cell membrane or tight junctions, will result in a decrease in ADC. As a result, tumors usually show a reduced ADC compared to necrotic tumors or normal tissue, as viable tumors usually show the highest concentration of cellular membrane and epithelial tight junctions because of higher cellular content.[5,6] The situation in metastatic lymph nodes is not as well established, with most reports showing lower ADC values with metastatic tumors compared to normal lymph nodes.[7-9]

In essence, these functional imaging methods detect tumor parameters, including cellular proliferation and possibly cellularity, and utilize these in the differentiation between malignant and normal tissue. Apart from these functional imaging techniques, these tumor parameters can also be measured by evaluating the various biological mediators. As our understanding of tumorigenesis increases, more biomarkers are being identified as important mediators for tumor growth. These markers can be detected as intracellular protein over expression by immunohistochemistry (IHC). More important, the advent of targeted therapy in the management of cancer patients has resulted in highly specific drug therapy directed at some tumor-specific protein

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Table 1
List of the protein markers used for immunohistochemistry

	Antibody	Company	Origin	Cat. #	Mono/polyclonal	Dilution
1	HER2	Dako	Denmark	A0485	Polyclonal	1:300
2	EGFR	Dako	Denmark	M7239	Monoclonal	1:30
3	VEGF	Dako	Denmark	M727329	Monoclonal	1:100
4	COX-2	Zymed	US	18-7379	Monoclonal	1:100
5	Bak	Santa Cruz	US	H-211	Polyclonal	1:100
6	Bax	Santa Cruz	US	P-19	Polyclonal	1:100
7	Bcl-2	Santa Cruz	US	C-2	Monoclonal	1:100
8	IL6	Abcam	UK	ab6672	Polyclonal	1:100
9	IL8	Biosource	US	AHC0782	Monoclonal	1:100
10	IL10	Abcam	UK	ab5132	Polyclonal	1:100

HER2, human epidermal growth factor receptor 2; *EGFR,* epidermal growth factor receptor; *VEGF,* vascular endothelial growth factor; *COX-2,* cyclooxygenase 2; *IL,* interleukin.

biological markers, and some of these IHC biological markers are now routinely evaluated in the assessment of specific tumors, e.g., CD117 (C-kit) in gastrointestinal stromal tumors, and human epidermal growth factor receptor 2 (HER2) in breast cancers. In HNSCC, protein markers as detected by IHC markers are associated with tumor progression from dysplasia to cancer,[10] stimulation of invasion,[11] and poor prognosis.[12,13] In HNSCC, the roles played by epidermal growth factor receptor (EGFR) and HER2 are fairly well known, whereas others, such as interleukins (ILs), cyclooxygenase (COX), vascular endothelial growth factor (VEGF), and the Bcl-2 family of proteins (Bcl-2, Bak, Bax), are also thought to be important.

In the current study, we evaluated a cohort of patients with HNSCC and correlated two radiological parameters obtained from in vivo MRI, namely Cho/Cr (derived from MRS) and ADC (derived from DWI), with a range of protein markers as assessed by IHC performed on the histological specimens.

Materials and Methods

Patients

Patients with previously untreated HNSCC enrolled in a functional MRI study approved by the local ethics committee and with informed patient consent were recruited for the study. Those with a successful MRS or DWI study of a primary tumor or metastatic node together with IHC of the histological specimen were included in the study.

Magnetic Resonance Imaging

MR examinations were performed on a 1.5-T whole-body system (Intera-NT, Best, The Netherlands) to obtain conventional images and functional images using MRS and DWI. MRS was performed by placing a volume of interest over the tumor identified on the conventional MRI. Water-suppressed spectra were acquired using the point-resolved spectroscopic sequence (TR/TE 2000/136 ms) to obtain 64 signal averages at a spectral bandwidth of 1000 Hz. Spectral analysis was performed in the time-domain. Briefly, after the removal of residual water (4.65 ppm) and lipid peaks (chemical shift range of 0.90-2.02 ppm) from the free induction decay, Cho and Cr peak amplitudes were determined. The choline level was expressed as a ratio against creatine to produce a choline/creatine ratio (Cho/Cr).

DWI was performed using a fat-suppressed diffusion-weighted spin-echo single-shot echo-planar imaging sequence. Briefly, this imaging sequence had 11 axial slices, 4.4 cm thick, and six diffusion-weighting, or b, values. ADC was calculated from multiple data points using a six-point regression method at b values of 0, 100, 200, 300, 400, and 500 s/mm^2. The ADC was measured by drawing a region of interest around the largest area of solid tumor on a single slice.

Immunohistochemistry (IHC) Assessment

Histological analysis was performed in the primary tumors that were excised. All the samples were fixed in buffered formalin and routinely processed. One representative slide from each case was selected for IHC staining for the antibodies (Table 1).

For the IHC staining, the primary antibodies were detected by biotinylated secondary antibodies. The signals were then amplified by avidin-biotin complex formation and developed with diaminobenzidine followed by hematoxylin counterstaining.

In some cases, the quantity of the archival material was limited and not all stains could be performed. All the immunohistochemical sections were scored by two of the authors in a blinded fashion, unaware of the imaging results. For EGFR and HER2, the staining assessed was membranous. For the other markers, namely VEGF, COX-2, Bak, Bax, Bcl-2, IL6, IL8, and IL10, the staining assessed was cytoplasmic. All slides were assessed for the staining intensity (0-3: no staining, 0; weak staining, 1; moderate staining, 2; strong staining, 3) and the percentage of tumor cells showing staining (0-100%). These two parameters were multiplied to give a composite score.