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ScienceDirect
Annals of Diagnostic Pathology 14 (2010) 23–29
Annals of DIAGNOSTIC PATHOLOGY

Clinicopathological implications of expressions of hypoxia-related molecules in esophageal superficial squamous cell carcinoma

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Abstract This study was conducted to clarify whether or not expressions of hypoxia-related molecules would have clinicopathological significance in squamous cell carcinoma (SCC) of the esophagus. Expressions of hypoxia inducible factor-1 alpha (HIF-1α), glucose transporter 1 (GLUT-1) and RAC-1 were immunohistochemically analyzed in 96 surgically resected SCCs at pT1b (sm1, 12 cases; sm2, 35 cases; sm3, 49 cases). They were divided into a lymph node metastasis (LNM)–positive group composed of 44 cases and an LNM-negative group composed of 52 cases. Immunohistochemical profiles were estimated based on the staining extent (score: 1+, 2+, 3+) and intensity (score: 1+, 2+, 3+). A significant expression pattern was found in the nucleus for HIF-1α, cell membrane for GLUT-1 and cytoplasm for RAC-1. The cases were categorized into a high score group (total score of 4 or more) and a low score group (total score of 3 or less) in each maker, respectively. A comparison made between the LNM-positive group and the LNM-negative group showed that the proportion of cases with a high score was larger in the LNM-positive group than in the LNM-negative group (HIF-1α, $P = .02$; GLUT-1, $P = .008$; RAC-1, $P = .001$). Among them, HIF-1α was found to be significantly related to the disease-free survival ($P = .019$) and overall survival ($P = .034$) as well as LNM (disease-free survival, $P = .030$; overall survival, $P = .030$). The multivariate analysis demonstrated that the HIF-1α expression would be an independent indicator for prognosis. In the superficial SCCs of the esophagus, GLUT-1 and RAC-1 may be involved in LNM, and HIF-1α overexpression is expected to predict an unfavorable clinical outcome.

Keywords: Esophagus; Squamous cell carcinoma; HIF-1α; GLUT-1; RAC-1

1. Introduction

Most esophageal cancers are squamous cell carcinoma (SCC). Unlike gastric adenocarcinoma, esophageal SCC with submucosal invasion is not regarded as true early cancer because of its relatively poor clinical outcome [1]. Thus, the early cancer of the esophagus is defined as limited to the mucosa irrespective of whether or not it has lymph node metastasis (LNM) [2].

Invasion and metastasis of malignant tumors leading to the poor prognosis are closely related to the degree of intratumoral hypoxia. The transcriptional activity of hypoxia inducible factor–1 (HIF-1) is determined by the expression of an HIF-1α subunit. HIF-1 plays a key role in the up-regulation of several hypoxia-related proteins involved in

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1092-9134/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.anndiagpath.2009.10.003

glucose transport, glycolysis, angiogenesis, erythropoiesis, inhibition of apoptosis, and monocyte-related inflammation [3]. Association of HIF-1α overexpression with histological character and acquisition of malignancy has also been reported [4,5]. Because malignant tumors usually reveal higher ratios of glucose uptake than normal tissues, previous studies have focused on the relationship between glucose transporter-1 (GLUT-1) overexpression and carcinogenesis, tumor development, unfavorable prognosis, and histological characterization [6-10]. Rho GTPases, 20- to 30-kd GTP-binding proteins defined as a subfamily of the Ras superfamily, are shown to regulate a wide spectrum of cellular functions [11] and to be involved in many critical phenomena such as tumorigenesis [12,13], metastasis [14,15], cell cycle [16,17], and apoptosis [18,19]. RAC-1 is considered to be one of the critical factors in Rho GTPase, which is hypoxia-inducible through the function of phosphatidylinositol 3′-kinase [20] and induces angiogenesis mediated by stabilization of HIF-1 [21]. RAC-1 activity is required for the activation of HIF-1α [22].

As for the esophageal SCC, some documents mention the association of higher expression of HIF-1α with an unfavorable clinical outcome [23,24] and with therapeutic resistance [25]. GLUT-1 expression profiles have been referred to as being related to biological behavior [26,27]. The cases used in these studies were comprised of esophageal SCCs at overall stages ranging from I to IV. Concerning RAC-1, to the best of our knowledge, there have been no reports examining the esophageal SCC. Using the surgically resected SCCs at pT1b without preoperative therapies, our study was designed to clarify whether or not a series of hypoxia-related factors such as HIF-1α, GLUT-1, and RAC-1 would be related to the clinical outcome.

2. Materials and methods

2.1. Patient selection

Out of the esophageal SCCs which were surgically resected at the Kanagawa Cancer Center between 1985 and 2006, the cases used in this study were selected based on the following conditions (Table 1): the carcinoma invasion was limited to up to the submucosa, staged at pT1b according to the Japanese Classification of Esophageal Cancer [2]; neither preoperative chemotherapy nor irradiation was performed; and regional lymph nodes dissection was made (range, 10-95; mean, 42.3; SD, 17.7). Postoperative chemotherapy was performed for the cases with LNM. Informed consent was obtained for all the patients examined in this study.

2.2. Immunohistochemistry

Formalin-fixed and paraffin-embedded tissue blocks from the primary lesion were cut into 4-μm sections. Immunohistochemical analysis of HIF-1α, GLUT-1, and RAC-1 expressions was performed using a representative section of the primary lesion. Sections were deparaffinized, and endogenous peroxidase activity was quenched with 0.3% hydrogen peroxidase. Heat-induced antigen retrieval was applied using an autoclave in citrate buffer (10 mmol/L, pH 6.0) as follows: at 121°C for 15 minutes for HIF-1α and RAC-1, and at 95°C for 15 minutes for GLUT-1. Sections were incubated with the following primary antibodies: HIF-1α (1:60, 4°C overnight, clone H1α67, Novus Biologicals, Littleton, CO); GLUT-1 (1:50, 30 minutes at room temperature, polyclonal, rabbit, Dako, Glostrup, Denmark); and RAC-1 (1:500, 4°C overnight, clone 102/RAC-1, BD Biosciences, San Jose, Calif). After rinsing in phosphate buffer saline (10 mM/L, pH7.2), sections were incubated with the Envision Kit (Dako, Glostrup, Denmark) for 30 minutes at room temperature. The reaction products were visualized with diaminobenzidine tetrahydrochloride.

The HIF-1α, GLUT-1, and RAC-1 immunostainings were scored for the extent and intensity, respectively, as follows: extent (0, negative; 1+ [focal], <30%; 2+ [partial], 30-60%; 3+ [diffuse], >60%) and intensity (0, negative; 1+ [weak]; 2+ [moderate]; 3+ [strong]). After adding the scores for each case, the cases were divided into 2 groups: (low score [LS] group, score 0-3) and (high score [HS] group, score 4-6). The following staining profiles were regarded as significant: nuclear staining for HIF-1α, cytoplasmic or cell membrane staining for GLUT-1, cytoplasmic staining for RAC-1.

Table 1
Clinicopathological data of 96 SCCs of the esophagus at pT1b

Clinicopathological data	Case no.
Age	
≤62	46
>62	50
Sex	
Male	89
Female	7
Differentiation	
Well	11
Moderately	45
Poorly	40
Invasion depth	
sm1	12
sm2	35
sm3	49
Vascular invasion	
v+	28
v−	68
Lymphatic invasion	
ly+	39
ly−	57
Lymph node metastasis	
Positive	44
Negative	52

2.3. Statistical analysis

χ^2 Test was used for assessing the significant difference in variable clinicopathologic factors. The univariate and multivariate analyses were performed using the Cox