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Original contribution

Decreased Beclin-1 expression is correlated with the growth of the primary tumor in patients with squamous cell carcinoma and adenocarcinoma of the lung

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Summary Beclin-1 is a Bcl-2–interacting protein, and it may delay cell cycle progression and induce autophagy. The function and expression of Beclin-1 and Bcl-2 in squamous cell carcinoma and adenocarcinoma of the lung remain largely unknown. Herein, we investigated the expression of Beclin-1 and Bcl-2 in squamous cell carcinoma and adenocarcinoma of the lung. Tissue samples from 262 cases were used in this study. Immunohistochemical staining for Beclin-1 and Bcl-2 were conducted using a tissue microarray. In squamous cell carcinoma, Beclin-1 expression was strongly positive in 48 (28.6%) of 168 samples, it was moderately positive in 42 (25.0%) of 168 samples, and it was negative or weakly positive in 78 (46.4%) of 168 samples. In adenocarcinoma, Beclin-1 expression was strongly positive in 26 (27.7%) of 94 samples, it was moderately positive in 27 (28.7%) of 94 samples, and it was negative or weakly positive in 41 (43.6%) of 94 samples. Beclin-1 expression was inversely correlated with the tumor size and the primary tumor stage (pT) in both types of tumor. Especially, the TNM stage of adenocarcinoma was inversely correlated with Beclin-1 expression. Our results suggest that a progressively reduced Beclin-1 expression is correlated with the primary tumor growth of squamous cell carcinoma and adenocarcinoma of the lung.

1. Introduction

Autophagy is the principal endogenous pathway for the degradation and recycling of long-lived proteins and organelles. Autophagic cell death is generally considered a type II programmed cell death [1]. Autophagy is known to be involved in many physiopathologic processes such as cell development, aging, stress responses, immune responses, and cancer [2]. In particular, emerging evidence has correlated impaired autophagy with tumor progression, although the results collected to date in this regard have been somewhat ambiguous [2]. The human *beclin-1* gene, which is located on chromosome 17q21, has been previously identified as the mammalian orthologue of Atg6 [3,4]. The *beclin-1* gene is monoallelically deleted in 40% to 75% of the sporadic breast, prostate, and ovarian tumors [5-7]. Furthermore, Beclin-1 mutant mice suffer from a relatively high incidence of tumors, including mammary gland neoplasia, lymphomas, lung adenocarcinomas (ACs), and hepatocellular carcinomas [8,9]. Based on these findings,

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Beclin-1 may be a haploinsufficient tumor suppressor gene. A reduced expression of Beclin-1 has also been demonstrated in some human cancers, including glioblastomas, ovarian cancers, and esophageal cancers [10-12]. However, an increased expression of Beclin-1 has been reported in colorectal and gastric cancer cells as compared with that of their normal counterparts [13]. These discrepant results indicate that Beclin-1 performs different functions in different tissues. Thus far, Beclin-1 expression and its clinical implications in squamous cell carcinoma (SCC) and AC have yet to be thoroughly elucidated.

Beclin-1 was first identified in a yeast 2-hybrid screen as a Bcl-2–interacting protein [14]. As an antiapoptotic protein, Bcl-2 physically interacts with Beclin-1; and it remains possible that Bcl-2 might be associated with the regulation of autophagy. Pattingre et al [15] previously demonstrated that Bcl-2 not only functions as an antiapoptotic protein but it also functions as an anti-autophagy protein via an inhibitory interaction with Beclin-1. The biological significance of the interaction between Beclin-1 and Bcl-2 in lung cancer has yet to be thoroughly explored. Therefore, we evaluated Beclin-1 and Bcl-2 expressions in SCC and AC tissue via immunohistochemical analysis, using a tissue microarray, in relation to survival and other prognostic factors.

2. Materials and methods

2.1. Patients and tissue samples

Tissue samples from 168 cases of SCC and 94 cases of AC were used in the present study. All of the tumors were surgically resected at the Kyung Hee University Hospital from 1983 to 2006. For each case, 2 investigators (K. Y. Won and G. Y. Kim) reviewed all of the original hematoxylin and eosin–stained sections. The clinicopathologic variables were evaluated, including age, sex, the histologic grade, tumor size, the primary tumor (pT), and nodal (pN) and distant metastasis (M), the TNM stage group, vascular invasion, lymphatic invasion, and the status of the resection margin. The TNM stage was classified in accordance with the seventh edition of the AJCC cancer staging protocols [16]. The mean patient follow-up duration was 65.1 months (range, 0-274 months). Among a total of 262 patients, 145 (55.3%) patients died of disease and 86 (32.9%) patients remained alive on the day of starting the study. Thirty-one (11.8%) patients were lost during the follow-up period. The age of the patients ranged between 35 and 81 years (median age, 61.2 years). The tumor size ranged between 0.7 and 11.8 cm. Tumor size was divided into 3 groups (<3 cm, 3-6 cm, ≥6 cm).

2.2. Tissue microarray construction

Hematoxylin and eosin–stained sections of the formalin-fixed, paraffin-embedded tumor tissue blocks were screened to identify the viable and representative areas. The corresponding areas on the block were marked for tissue punching. The tissue microarrays were assembled using a commercially available manual tissue microarrayer (Quick-Ray; UNITMA Co, Ltd, Seoul, Korea). Briefly, 2 or 3 tumor cores with a diameter of 2.0 mm were punched from each tumor tissue block, and these cores were arrayed into 2 or 3 paraffin recipient blocks, respectively. We chose two or three 2.0-mm cores per case to increase the concordance rate between the immunohistochemistry results of the tissue microarrays and the whole sections. Cores from 168 SCCs and 94 ACs could be placed in each block. For each block, hematoxylin and eosin staining was performed to verify the tumor cell content. The cases with stromal tissue only or insufficient cancer tissue in all the cores were excluded from the analysis. Serial sectioned slides were produced, and hematoxylin and eosin staining was done.

2.3. Immunohistochemical staining

Immunohistochemistry was conducted on the 4-μm tissue sections using the Bond Polymer Intense Detection system (Vision BioSystems, Victoria, Australia) according to the manufacturer's instructions with minor modifications. In brief, 4-μm sections of formalin-fixed, paraffin-embedded tissue were deparaffinized using Bond Dewax Solution (Vision BioSystems), and an antigen retrieval procedure was conducted using Bond ER Solution (Vision BioSystems) for 30 minutes at 100°C. The endogenous peroxidase was quenched by incubating the tissues with hydrogen peroxide for 5 minutes. The sections were incubated for 15 minutes at ambient temperature with primary polyclonal antibodies for Beclin-1 (1:100, Abcam, Cambridge, UK) and Bcl-2 (1:200, clone 124, Dako, Glostrup, Denmark) using a biotin-free polymeric horseradish peroxidase-linker antibody conjugate system in a Bond-max automatic slide stainer (Vision BioSystems). The nuclei were counterstained with hematoxylin.

2.4. Evaluation of the immunohistochemical staining

The expression of Beclin-1, as determined by immunohistochemical staining, appeared as fine granular and diffuse cytoplasmic staining with sporadic nuclear staining. A Bcl-2 expression was noted in the cytoplasm of the tumor cells. The immunoreactivity of the granulosa-lutein cells in the ovary cells and in the normal ductal epithelium in the breast tissue was used as a positive control for Beclin-1 [17]. Small lymphocytes in the mantle zone functioned as a positive control for the Bcl-2 expression. Beclin-1 and Bcl-2 expressions were analyzed with a semiquantitative scoring method. The score was calculated according to the intensity and proportion of the immunoreactivity. The intensity score was determined as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The proportion score was determined as 0 (no staining), 1 (<30% positivity