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Original contribution

Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer

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Summary The human *beclin-1* gene, located on chromosome 17q21, has been identified as the mammalian orthologue of Atg6 (autophagy-related gene) and may be a haploinsufficient tumor suppressor gene. The function and expression of beclin-1 in human breast cancer are largely unknown. We investigated the expression of beclin-1 and bcl-2 in human breast cancer. Tissue samples from 125 cases of invasive breast cancer were used for the present study. Immunohistochemical staining for beclin-1 and bcl-2 was evaluated using tissue microarray, then the 2 proteins were correlated with clinicopathologic parameters. Positive beclin-1 expression and bcl-2 expression in breast cancer tissue were observed in 53 cases (42.4%) and 48 cases (38.4%), respectively. Beclin-1 expression was inversely correlated with bcl-2 expression in breast cancer tissue (P = .035). Beclin-1 expression significantly correlated with nuclear pleomorphism and mitotic count. Bcl-2 expression in breast cancer tissue significantly correlated with histologic grade, tubule formation, nuclear pleomorphism, mitotic count, estrogen receptor, and distant metastasis. Our results suggest that beclin-1 might play a role in the inhibition of the development of breast cancer and that inhibition might be due to an interaction with bcl-2 protein.

1. Introduction

Autophagy is a catabolic process where cells self-digest intracellular organelles. Autophagy is a genetically controlled process that results in the targeting of cellular proteins and organelles to lysosomes for degradation [1,2]. This process may serve to regulate normal turnover of organelles and to remove organelles with compromised function to maintain homeostasis. Autophagy is mediated by a set of evolutionarily conserved gene products originally discovered in yeast, which are called autophagy-related genes [3].

The human *beclin-1* gene, located on chromosome 17q21, has been identified as the mammalian orthologue of Atg6 (autophagy-related gene) [4,5]. Liang et al [5] have shown that beclin-1 can function as a negative regulator of mammary cell growth and tumorigenesis as well as in the induction of autophagy in breast cancer cells. In addition, in the study, there was a significant loss of beclin-1 protein

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expression in 56% of human breast cancer cells as compared to normal breast epithelial cells [5]. The *beclin-1* gene is monoallelically deleted in 40% to 75% of sporadic breast, prostate, and ovarian tumors [6-8]. Furthermore, beclin-1 mutant mice suffer from a high incidence of tumors, including mammary gland neoplasia, lymphomas, lung adenocarcinomas, and hepatocellular carcinomas [9,10]. Based on these findings, beclin-1 may be a haploinsufficient tumor suppressor gene. Decreased expression of beclin-1 has also been demonstrated in some human cancers including glioblastomas, ovarian cancers, and esophageal cancers [11-13]. However, increased expression of beclin-1 has been reported in colorectal and gastric cancer cells as compared to the normal counterparts [14]. These discrepant results suggest that beclin-1 has different roles in different tissues.

Beclin-1 was first identified in a yeast 2-hybrid screen as a bcl-2–interacting protein [15]. As the antiapoptotic protein, bcl-2, physically interacts with beclin-1, the possibility is raised that bcl-2 might be related to the regulation of autophagy. Pattingre et al [16] showed that bcl-2 functions not only as an antiapoptotic protein but also as an antiautophagy protein by an inhibitory interaction with beclin-1. Expression of beclin-1 and an association with bcl-2 expression, as well as any correlation between beclin-1 expression and clinicopathologic factors or outcome in human cancers, have not been well characterized.

We investigated the expression of beclin-1 and bcl-2 in human breast cancer tissue by 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 125 cases of invasive breast cancer were used for the present study. All of the neoplasms were surgically resected at Kyung Hee University Hospital from 1999 to 2006. For each case, 2 investigators (WKY and LSJ) reviewed all of the original hematoxylin and eosin–stained sections. Two or 3 representative cores, 3 mm in diameter, were taken from a representative area of each paraffin-embedded tumor tissue from which tissue microarray slides were constructed. The mean patient follow-up duration was 63.9 months (range, 7-103 months). For 125 patients, 20 patients died of disease and 102 patients were alive on the day of the study. Three patients were lost during the follow-up period. The age of patients ranged from 23 to 71 years (median age, 49.4 years). Tumor size ranged from 0.5 to 12 cm (median size, 3.02 cm). Grade as classified by the Nottingham Modification of the Bloom-Richardson system was as follows: there were 33 grade 1 cases (26.4%), 60 grade 2 cases (48.0%), and 32 grade 3 cases (25.6%).

2.2. Immunohistochemical staining

Immunohistochemistry was performed on 4-μm-thick 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-thick sections of formalin-fixed, paraffin-embedded tissue were deparaffinized by the use of Bond Dewax Solution (Vision BioSystems) and an antigen retrieval procedure was performed using Bond ER Solution (Vision BioSystems) for 30 minutes at 100°C. Endogenous peroxidases were quenched by incubation of tissue with hydrogen peroxide for 5 minutes. Sections were incubated for 15 minutes at ambient temperate with primary polyclonal antibodies for beclin-1 (1:100, Abcam, Cambridge, United Kingdom) 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). Nuclei were counterstained with hematoxylin.

2.3. Evaluation of immunohistochemical staining

The expression of beclin-1 as determined by immunohistochemical staining appeared as fine granular and diffuse cytoplasmic staining with occasional nuclear staining. Bcl-2 expression was seen in the cytoplasm of breast cancer cells. Immunoreactivity of granulose-lutein cells in ovary cells and in the normal ductal epithelium in breast tissue was used as a positive control for beclin-1. A positive control for bcl-2 expression was small lymphocytes in the mantle zone. Immunohistochemical staining for beclin-1 and bcl-2 was evaluated according to intensity and proportion. 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 1 (<30% of tumor cells) and 2 (≥30% of tumor cells). The intensity score and proportion score were multiplied together for a total score. Total scores were as follows: 0 to 1 (negative) and 2 to 6 (positive). All slides were evaluated independently by 2 investigators (WKY and LSJ) without knowledge of the identity of the patient and clinical outcome.

2.4. Statistical analysis

The Pearson's χ^2 test was used to evaluate the association between beclin-1 expression and bcl-2 expression and several clinicopathologic variables. The Kaplan-Meier method was used to determine the probability of survival, and the data were analyzed by the use of the log-rank test. Overall survival was defined as survival from the date of surgery to the date of death due to cancer. The Cox proportional hazards model was used for multivariate analysis of prognostic factors. A P value of less than .05 was considered as significant.