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Original contribution

Senescence in intraductal papillary mucinous neoplasm of the pancreas☆,☆☆

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Summary Intraductal papillary mucinous neoplasm of the pancreas is attracting attention as a precursor lesion of the invasive ductal adenocarcinoma, whereas it has been reported that some intraductal papillary mucinous neoplasms do not display progression to malignancy and remain almost unchanged in size and morphology. Recent studies have reported that oncogene-induced senescence has been observed in neoplasms, especially in premalignant lesions, and that it can play an important role in preventing malignant progression. To clarify the presence of senescence in intraductal papillary mucinous neoplasms, we analyzed the expression of several markers of senescence. The intraductal papillary mucinous neoplasms evaluated in this study were classified into 4 groups according to the degree of dysplasia. Senescence-associated β-galactosidase activity and senescence-associated heterochromatin foci formation were investigated in 33 cases of intraductal papillary mucinous neoplasms and 6 normal controls. Immunohistochemical analysis of $p16^{INK4a}$ and $p15^{INK4b}$ was performed in 158 cases of intraductal papillary mucinous neoplasms and 10 normal controls. In the normal controls, neither senescence-associated β-galactosidase activity nor senescence-associated heterochromatin foci formation was observed. Most of the normal epithelia were negative for either $p16^{INK4a}$ or $p15^{INK4b}$. For all 4 markers, the percentages of positive cases reached a peak in intraductal papillary mucinous neoplasm with low-grade dysplasia and showed significant decreasing trends in the transition from intraductal papillary mucinous neoplasm with low-grade dysplasia to intraductal papillary mucinous neoplasm with an associated invasive carcinoma. Our results indicate that senescence is induced in the early stage of intraductal papillary mucinous neoplasm and gradually attenuated according to the progression. It is suggested that senescence plays a role in preventing malignant progression of intraductal papillary mucinous neoplasm.

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1. Introduction

Invasive ductal adenocarcinoma of the pancreas is the most lethal cancer of the digestive system. More than half of all patients have unresectable lesions at the time of diagnosis. Even if the lesion is resectable, it is usually found in the advanced stages, and the 5-year survival rate is less than 20%.

Intraductal papillary mucinous neoplasm (IPMN) is attracting attention as a precursor of invasive ductal adenocarcinoma of the pancreas. It exhibits dilated main/branch pancreatic ducts, an intraductal papillary structure, and massive extracellular mucin production. The World Health Organization divides IPMN into 4 groups based on the degree of dysplasia and presence of invasive carcinoma components: IPMN with low-grade dysplasia (*l*-IPMN), IPMN with intermediate-grade dysplasia (*i*-IPMN), IPMN with high-grade dysplasia (*h*-IPMN), and IPMN with an associated invasive carcinoma (*inv*-IPMN) [1]. Because IPMNs often exhibit a variety of dysplasia in the same lesion, it is widely accepted that they show a sequential progression pattern from *l*-IPMN to *inv*-IPMN [2]. On the other hand, long-term observation of branch duct IPMNs has revealed that some benign IPMNs do not display progression to malignancy and remain almost unchanged in size and morphology [3,4].

Senescence is a permanent cell cycle arrest induced by telomere shortening that can be caused by an accumulation of cell doublings, DNA damage due to irradiation, oxidative stress, or activation of oncogenes. Although this phenomenon was originally reported in vitro, it has also been observed in vivo [5]. Recent studies have reported that oncogene-induced senescence has been observed in neoplasms, especially in premalignant lesions, and plays an important role in preventing malignant progression [6,7]. To identify senescence, several markers have been proposed. Senescence-associated β-galactosidase (SA-β-gal) activity is widely used for detection of senescent cells in vitro and in vivo [5]. Narita et al [8] described that senescence-associated heterochromatin foci (SAHF) formation was observed in senescent cells. Collado et al [7] introduced $p16^{INK4a}$ and $p15^{INK4b}$ as immunohistohemical markers of oncogene-induced senescence.

Several molecules, including $p16^{INK4a}$, $p14^{Arf}$, and $p21^{Waf1}$, have been linked to oncogene-induced senescence. Recently, DNA damage checkpoint activation has been proposed to be involved in the induction of oncogene-induced senescence both in vitro and in vivo [9,10]. We have previously reported that DNA damage checkpoint activation occurred in the early stage of IPMNs [11]. Considering that long-term stasis exists in some IPMNs, the existence of senescence in premalignant IPMNs is suggested.

To determine whether senescence takes place during tumorigenesis and is involved in the progression of IPMNs, we investigated the expression of several markers of senescence in each grade of dysplasia of IPMNs.

2. Materials and methods

2.1. Samples

Original hematoxylin and eosin slides of 158 patients with IPMN registered in the files of the Department of Anatomic Pathology, Kyushu University, between 1986 and 2007 were reviewed and classified into 4 groups according to the World Health Organization classification: *l*-IPMN, *i*-IPMN, *h*-IPMN, and *inv*-IPMN [1]. *h*-IPMN and *inv*-IPMN were considered malignant. A slide containing the lesion with the highest degree of dysplasia for each case was selected as the representative section. Formalin-fixed, paraffin-embedded (FFPE) blocks of all representative sections were available for immunohistochemical analysis. We also subclassified these cases according to macroscopic involvement of the pancreatic duct system (macroscopic subtype) and microscopic morphology (morphological subtype). In macroscopic subtype, IPMNs were classified into branch duct and main duct types [1]. Combined type was included in main duct type. In the morphological subtype, IPMNs were divided into 4 types based on architectural and cell differentiation patterns: gastric, intestinal, pancreatobiliary,

Table 1 Subtype and degree of dysplasia

	l-IPMN	*i*-IPMN	*h*-IPMN	*inv*-IPMN	Total
Macroscopic subtype					
Branch duct	51	24	18	15	108
Main duct	12	11	10	17	50
Morphological subtype					
Gastric	58	16	2	6	82
Intestinal	5	19	15	10	49
Pancreatobiliary	0	0	2	9	11
Oncocytic	0	0	4	1	5
Unclassified	0	0	5	6	11