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Original contribution

Dual gain of *HER2* and *EGFR* gene copy numbers impacts the prognosis of carcinoma ex pleomorphic adenoma☆,☆☆

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Summary We investigated the potential roles of HER2 and EGFR and evaluated their prognostic significance in carcinoma ex pleomorphic adenoma (CXPA). We analyzed HER2 and EGFR overexpression status using immunohistochemistry (IHC) and gene copy number gain by chromogenic in situ hybridization (CISH) in 50 cases of CXPA (40 ductal-type and 10 myoepithelial-type CXPAs). Salivary duct carcinoma was the most common histologic subtype of malignant component (n = 21). Immunohistochemistry positivity and chromogenic in situ hybridization positivity were closely correlated in both HER2 and EGFR. *HER2* CISH positivity (mostly gene amplification) and *EGFR* CISH positivity (mostly gene high polysomy) were present in 19 (40%) and 21 (44%) cases, respectively, and were each significantly correlated with poor outcome (P = .0009 and P = .0032, respectively). Dual gain of *HER2* and *EGFR* gene copy numbers was present in 11 cases (23%) and was the most aggressive genotype. *HER2* CISH positivity was more frequently present in ductal-type CXPAs (47%) than in myoepithelial-type CXPAs (10%), whereas the prevalence of *EGFR* CISH positivity was similar in both histologic subtypes (42% and 50%, respectively). Our results suggest that *HER2* and *EGFR* gene copy number gains may play an important role in the progression of CXPA, in particular ductal-type CXPAs. *HER2* CISH-positive/*EGFR* CISH-positive tumors may be the most aggressive subgroup in CXPA. The molecular subclassification of CXPA based on the HER2 and EGFR status may be helpful for prognostic prediction and decisions regarding the choice of therapeutic strategy.

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1. Introduction

Carcinoma ex pleomorphic adenoma (CXPA) of the salivary gland is a malignant tumor that develops in or from a benign pleomorphic adenoma (PA), and it is considered one model of an adenoma-carcinoma sequence in salivary glands. CXPAs comprise approximately 4% of all salivary tumors and 12% of all salivary malignancies [1,2]. Approximately 6% of PAs are estimated to undergo malignant transformation and turn into CXPAs.

Fig. 1 provides a schema of the stepwise progression of CXPA. CXPAs are mainly classified into 2 groups by the phenotype of malignant components: the ductal type (75% of CXPAs) and the myoepithelial type (25%) [3]. Ductal-type CXPAs presumably develop as follows. The carcinoma cells initially replace the inner layer of ducts while retaining the peripherally located intact myoepithelial layer (intraductal carcinoma or carcinoma in situ), and then the carcinoma cells break through the myoepithelial layer but not the capsule of the preexisting PA (intracapsular carcinoma), and they eventually invade beyond the fibrous capsule to the extracapsular area (extracapsular carcinoma) [4-6]. Histologically, most intraductal ductal-type CXPAs are equivalent to the intraductal component of salivary duct carcinoma (SDC), and the intracapsular or extracapsular invasive ductal-type CXPAs can be subclassified into the SDC type and adenocarcinoma not otherwise specified (ADC-NOS) type [3,5,7]. Myoepithelial-type CXPAs histologically correspond to myoepithelial carcinomas (MYECs), of which the extent of invasion can be classified as intracapsular or extracapsular; intraductal "myoepithelial carcinoma" does not exist.

To date, several molecules, notably p53, S100P, and human epidermal growth factor receptor 2 (HER2), have been suggested to play a role in the malignant transformation of PA and the progression of CXPA [5,7-11].

HER2 and epidermal growth factor receptor (EGFR) are receptor tyrosine kinases that play important roles in the development and progression of various types of cancer [12,13]. Tyrosine kinase inhibitors against HER2 or EGFR are currently in clinical use for the treatment of breast, lung, and gastrointestinal cancers.

HER2 overexpression and *HER2* gene amplification have each been reported to be associated with the aggressiveness and poor outcome of several salivary gland malignancies, including mucoepidermoid carcinoma (MEC), SDC, and ADC-NOS [5,11,14-16]. In CXPAs, HER2 overexpression or *HER2* gene amplification has been demonstrated in 20% to 60% of the cases by several investigators [7,10,11]. In our recent study using chromogenic in situ hybridization (CISH), *HER2* gene amplification was observed in one-half of ductal-type CXPAs, in which *HER2* gene amplification was essentially maintained from the noninvasive (intraductal) component to the invasive component [5]. *HER2* gene amplification was a poor prognostic factor in ductal-type CXPAs. The molecular mechanisms underlying the progression of CXPA, other than *HER2* gene amplification, have not been established.

EGFR overexpression has been demonstrated in approximately 25% of salivary gland malignancies, including 25% to 70% of SDCs and 30% to 60% of MECs [16-18]. An

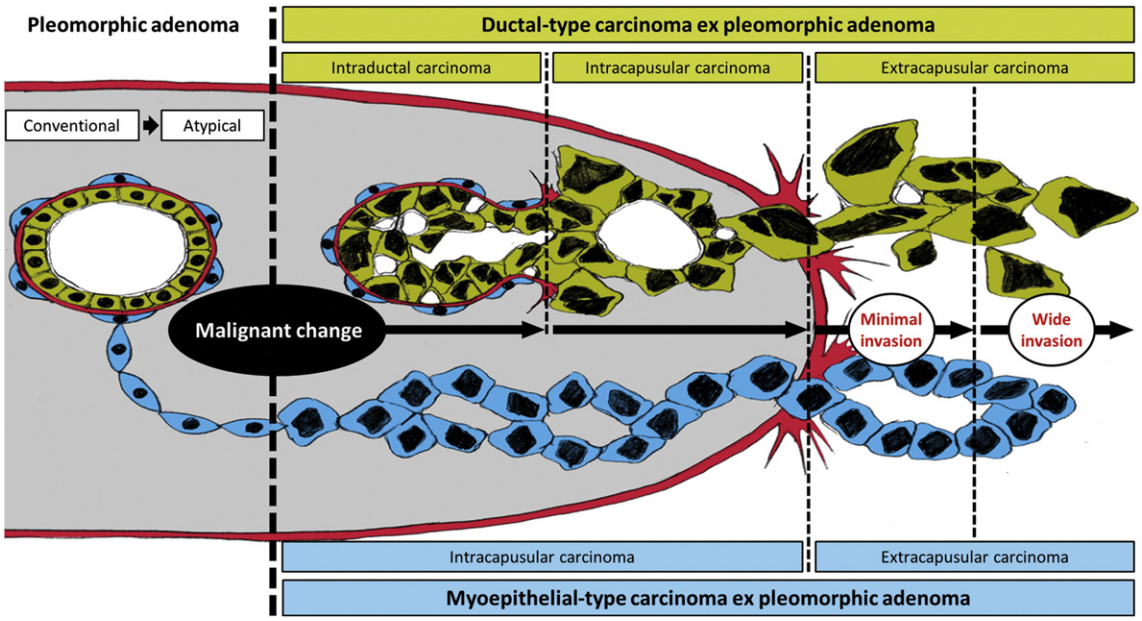

Fig. 1 Schema of the stepwise progression of CXPA, including ductal-type and myoepithelial-type CXPAs.