Roles of N-cadherin in Head and Neck Squamous Cell Carcinoma

Abstract

Head and neck squamous cell carcinoma (HNSCC) is one of the most common types of human cancer, with an annual incidence of more than 500,000 cases worldwide. Like most epithelial cancers, HNSCC develops through the accumulation of multiple genetic and epigenetic alterations in a multistep process. Metastasis is the most important prognostic factor. Therefore, attempts to identify the genes involved in the invasion and/or metastasis are pivotal for the early prediction of HNSCC behaviors. During tumor progression, cancer cells undergo dramatic changes in the expression profile of adhesion molecules resulting in detachment from original tissue and acquisition of a highly motile and invasive phenotype. A hallmark of this change is referred to as epithelial-mesenchymal transition (EMT). A key to EMT is the reduction of cell-cell adhesion by transcriptional repression of cadherin. The loss of E-cadherin and the gain of N-cadherin expression are known as the “cadherin switching”. Cadherin switching is a major hallmark of EMT. Although E-cadherin is well documented as an invasive suppressor for cancer cells, aberrant expression of N-cadherin is considered as association with increased tumor progression in certain cancers. Therefore, I investigated whether N-cadherin expression was associated with the progression of HNSCC. I examined the expression of N-cadherin in 80 HNSCC cases by immunohistochemistry and its correlation with clinico-pathological findings. High expression of N-cadherin was observed in 52 of 80 HNSCC cases and was significantly correlated with malignant behaviors. Cadherin switching was found in 30 of 80 HNSCC cases and was well correlated with histological differentiation, pattern of invasion and lymph node metastasis in HNSCC cases. Moreover, I examined the expression of N-cadherin and E-cadherin by RT-PCR in 16 HNSCC cell lines to confirm the immunohistochemical findings. N-cadherin expression was observed in 7 of 16 HNSCC cells, and cadherin switching was observed in 2 HNSCC cells. Interestingly, HNSCC cells with cadherin switching have EMT features.

Spindle cell carcinoma (SpCC) occurs mainly in the upper aerodigestive tract. SpCC is thought as a variant of squamous cell carcinoma, which shows biphasic proliferation of conventional squamous cell carcinoma component and malignant spindle
shape cells with sarcomatous appearance. It is generally accepted that the sarcomatoid cells are derived from squamous cells. I referred SpCC as an EMT type of squamous cell carcinoma. That is the reason why we also investigated the hypothesis that spindle cell phenotype might be related to cadherin switching. I used 2 SpCC cell lines that previously established in our department. The diagnosis of SpCC was made on the basis of histologic and immunohistochemical findings. Similar to the primary tumor, these cell lines showed the expression of cytokeratins and vimentin. Moreover, loss of E-cadherin expression was observed. In 2 SpCC cell lines, I examined the expression of N-cadherin. These cells showed high expression of N-cadherin in their cytoplasm. Thus, cadherin switching was observed in SpCC cell lines. Moreover, I examined the expression of N-cadherin in SpCC cases by immunohistochemistry. Although non-neoplastic squamous epithelium was completely negative, high expression of N-cadherin was observed in 10 of 15 SpCC cases. All cases showed reduced expression of E-cadherin in comparison with non-cancerous epithelial cells. Interestingly, 6 of 7 SpCC cases with metastasis showed high expression of N-cadherin.

These findings prompted me to perform a series of in vitro and in vivo studies, with the hypothesis that N-cadherin is crucial in HNSCC progression. More recently it has been illustrated that the IDPVNGQ sequence of the forth extracellular of N-cadherin interacted with HAV sequence of FGF receptor (FGFR) to promote several malignant behaviors. A 69 amino acid portion of the forth extracellular domain was necessary for EMT and increase motility in squamous epithelial cell. To know the utility of N-cadherin as a therapeutic target for HNSCC, I examined the effects of N-cadherin antibody which binds specific the forth extracellular domain of N-cadherin in HNSCC cells with EMT features (HOC313). I found that N-cadherin antibody inhibited the proliferation and the invasion in HOC313 cells. These cells showed morphologic changes from a spindle shape to cobblestone-like shape. I noted that blocking function of N-cadherin may induce mesenchymal to epithelial transition (MET). Interestingly, MET induction suppressed cell growth and invasion. Next, I examined expression of several mesenchymal markers in N-cadherin antibody treated HOC313 cells. I found the reduced expression of Snail1 and vitronectin in HOC313 cells with N-cadherin antibody treatment. Moreover, reduced expression of matrix metalloproteinases (MMPs) such as MMP3, MMP7, MMP9, MMP10, MM12 and MMP13 were observed after N-cadherin antibody treatment.

In conclusion, I suggest that N-cadherin may play an important role in malignant behaviors of HNSCC, and that cadherin switching might be considered as a discrete critical event in EMT and metastatic potential of HNSCC. Blocking function of N-cadherin can be a therapeutic target for HNSCC.