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FOXA1 in HPV associated carcinomas: Its expression in carcinomas of the head and neck and of the uterine cervix

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ABSTRACT

Background: FOXA1 is a major transcription factor involved in the action of human papilloma virus (HPV). However, it has been never studied in HPV-associated tumors.
Aim of the study: To investigate its expression in cervical and head and neck tumors.
Material and methods: 63 cervical carcinomas/dysplasias and 152 head and neck squamous cell carcinomas (HNSCC) were immunohistochemically studied for the expression of FOXA1.
Results: 63.1% of cervical SCC and 40.7% of endocervical adenocarcinomas strongly expressed FOXA1. Most (90%) pre-invasive lesions (CIN3 and in situ adenocarcinomas) strongly expressed FOXA1 and this difference from invasive lesions was statistically significant ($p = 0.005$). No association with clinicopathological factors was found. 51.3% of HNSCC expressed FOXA1. In these tumors, FOXA1 expression was associated with the non-keratinizing morphology but not with the HPV/p16 status neither other clinicopathological features. Of normal structures, salivary glands, endocervical glands and basal/parabasal cell layer of squamous epithelium of both uterine cervix and head and neck mucosa, all strongly expressed FOXA1.
Conclusion: FOXA1 is expressed by basal cells of squamous epithelium, pre-invasion lesions of the uterine cervix and the head/neck and almost half invasive cervical and head/neck carcinomas, supporting its possible implication in HPV pathogenesis.

1. Introduction

Forkhead-box A1 transcription factor (FOXA1) is the major factor regulating differentiation of endoderm-derived organs during embryogenesis (Friedman and Kaestner, 2006). It is also implicated in the pathogenesis of hormone-dependent tumors like breast, endometrial and prostate cancer, as it is a transcription factor necessary for the binding of hormone receptors on chromatin (Carroll et al., 2005; Horimoto et al., 2014; Hurtado et al., 2011; Jin et al., 2014). We showed that regarding ovarian neoplasms, FOXA1 is expressed almost exclusively in mucinous and Brenner tumors, probably recapitulating in these tumors its role during endodermal histogenesis (Karpathiou et al., 2016a).

Apart from its role in hormone-dependent carcinogenesis and endoderm histogenesis, it has been shown in cell lines that FOXA1 is a transcription factor able to bind to HPV genome and thus a potential regulator of its transcription (Sichero et al., 2012). However, it has never been studied in HPV associated cancers.

The aim of the study was to investigate FOXA1 expression in HPV-associated lesions, namely cervical and head/neck carcinomas.

2. Material and methods

2.1. Study population

Sixty three specimens from 52 patients diagnosed with a cervical carcinoma/dysplasia and 152 specimens from 152 patients with head and neck squamous cell carcinoma were included in the study. Material used was either biopsies obtained before any chemo- or radiotherapy or surgical specimens of patients treated without neoadjuvant therapy. Clinical information was collected through the electronic medical records. Local ethics committee of the University Hospital of St-Etienne approved the study (DC-2015-2489).

Detailed demographics concerning the HNSCC cohort have been previously described (Karpathiou et al., 2016b). Briefly, 84.2% were

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male with a median age of 58.5 years at the time of diagnosis. Most tumors (94.8%) were diagnosed at an advanced stage. The median follow up was 24 months with 57.2% of the patients being alive at the time of the last follow up.

Patients with cervical lesions were diagnosed at an age range of 28 to 90 years (mean and median, 56.2 and 53 years, respectively). FIGO stage (available for 42 patients) was 0-II for 30 patients and III–IV for 12 patients. Two patients were lost to follow up. Overall survival for the 50 patients ranged from 1 to 200 months with a mean of 55.6 months and a median of 34 months. Nine of the patients (18%) died of the disease, while 41 were alive at the time of the last follow-up.

2.2. Histopathological evaluation

2.2.1. Head and neck squamous cell carcinomas

SCCs were divided into keratinizing (K-SCC) and non-keratinizing (NK-SCC) types, as previously suggested (Karpathiou et al., 2016b; Chernock et al., 2009; Gondim et al., 2016; Karpathiou et al., 2016c; Karpathiou et al., 2016d). The Brandwein-Gensler (BG) histologic risk assessment model was used as a histoprognostic system that estimates the worst pattern of invasion, the lymphocytic host response and the perineural invasion (Karpathiou et al., 2016b; Karpathiou et al., 2016c; Karpathiou et al., 2016d; Brandwein-Gensler et al., 2010; Brandwein-Gensler et al., 2005). Other histological features were also recorded: the absence or presence of large areas of necrosis, and the absence or presence of a marked fibroblastic reaction.

One hundred twenty two (122) patients were tested for HPV infection at the time of diagnosis. Four were positive. In order to augment sensitivity of HPV-detection, p16 expression (clone E6H4, CINtec), was also performed in the whole sample revealing 27 (18%) positive tumors. Of these, 20 were oropharyngeal and 7 were non-oropharyngeal tumors.

2.2.2. Carcinomas of the uterine cervix

The cohort includes 19 patients (36.5%) with an invasive SCC, 27 (51.9%) with an invasive adenocarcinoma and 6 (11.5%) with an adenocarcinoma in situ. Furthermore, 10 high grade intraepithelial lesions (CIN3) and another adenocarcinoma in situ were found in adjacent tissue. Invasive adenocarcinomas included one of endometrioid type, one minimal deviation adenocarcinoma (adenoma malignum) and 25 endocervical adenocarcinomas, not otherwise specified (NOS). All patients with cervical carcinomas, but the one with adenoma malignum, had a clinical history of HPV infection and p16 positive lesions. For adenocarcinomas, invasive and in situ, the Silva histological model (Diaz De Vivar et al., 2013) was used showing the presence of 9 pattern A tumors, 4 pattern B tumors and 14 pattern C tumors. Seven of the specimens were biopsies not evaluated by this system.

2.3. Immunohistochemical analysis

Four-μm thick full tumor sections were used for immunohistochemistry, which was performed using an automated staining system (Leica Biosystems, Newcastle Upon Tyne, UK). Positive immunoreactions were visualized using 3,3′-diaminobenzidine as the chromogenic substrate. Goat polyclonal antibody against FOXA1 [1:50, Santa Cruz Biotechnology, clone HNF-3α/β (C-20): sc-6553] was used as the primary antibody. FOXA1 positive breast carcinoma was used as positive control and the same tissue with omission of the primary antibody was used as negative control. Tumors were designated as FOXA1 positive when ≥5% of tumor cells were positive. Immunoreactivity of adjacent normal tissues was also recorded.

2.4. Statistical analysis

Data were analyzed using the StatView software (Abacus Concepts, Berckley Ca, USA). Relationship between two groups was investigated

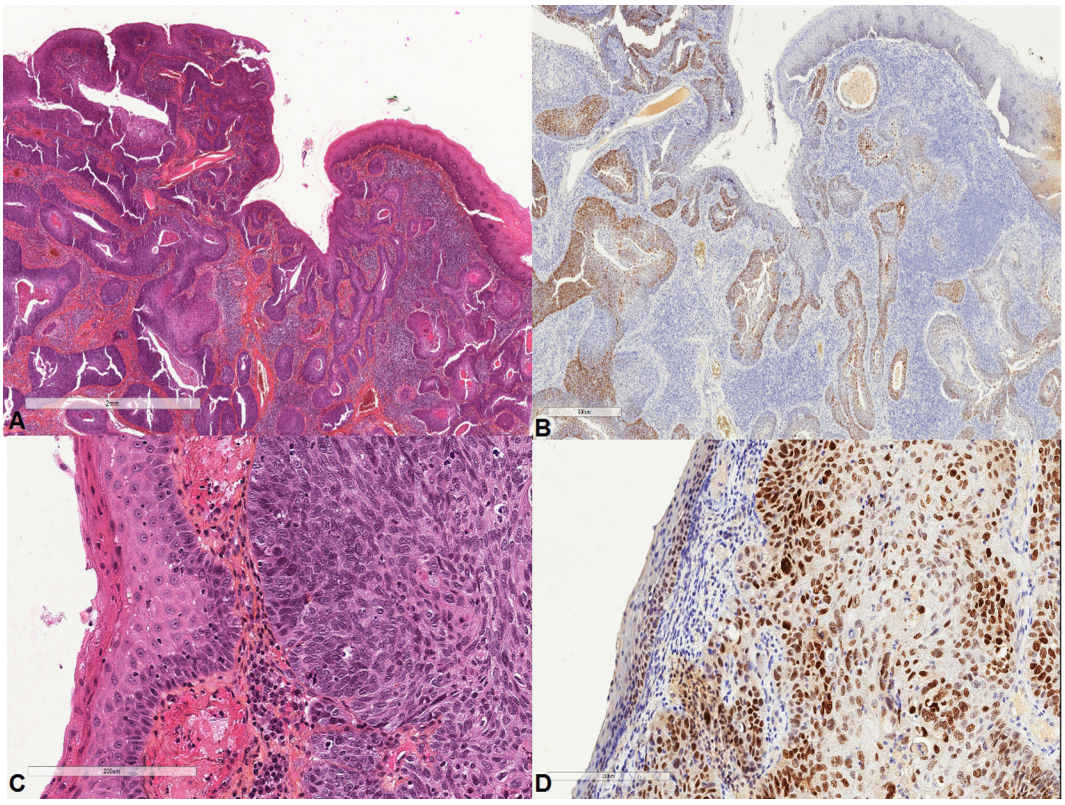

Fig. 1. FOXA1 expression in HNSCC. A. Non-keratinizing SCC (HES × 20). B. FOXA1 study of this tumor shows expression by the invasive lesion and the overlying dysplasia (×20). C. Another HNSCC with the overlying epithelium (HES × 200). D. Strong FOXA1 expression of the same tumor. Basal cells of the overlying epithelium also express FOXA1 (×200).