Acta Histochemica xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Co-expression of TTF-1 and neuroendocrine markers in the human fetal lung and pulmonary neuroendocrine tumors

Josip Miskovic[a], Zdrinko Brekalo[a], Katarina Vukojevic[b], Helena Radic Miskovic[c], Daniela Kraljevic[d], Jelena Todorovic[e], Violeta Soljic[e,f,*]

[a] Department of Surgery, University Hospital in Mostar, KraljaTvrtka bb, 88000 Mostar, Bosnia and Herzegovina
[b] Laboratory for Early Human Development, Department of Anatomy, Histology and Embryology, School of Medicine, University of Split, Soltanska 2, 21000 Split, Croatia
[c] Department of Neonatology, University Hospital in Mostar, Kralja Tvrtka bb, 88000 Mostar, Bosnia and Herzegovina
[d] Department of Pediatrics, University Hospital in Mostar, Kralja Tvrtka bb, 88000 Mostar, Bosnia and Herzegovina
[e] Department of Pathology, Cytology and Forensic Medicine, University Hospital in Mostar, Kralja Tvrtka bb, 88000 Mostar, Bosnia and Herzegovina
[f] Department of Histology and Embryology, School of Medicine, University of Mostar, Bijeli brijeg bb, 88000 Mostar, Bosnia and Herzegovina

ARTICLE INFO

Article history:
Available online xxx

Key words:
Fetal lung
TTF-1
Neuroendocrine cell
Markers
Tumors

ABSTRACT

The expression pattern of thyroid transcription factor 1 (TTF-1) and neuroendocrine markers, neuron cell adhesion molecule (NCAM; CD56), chromogranin A (CgA) and synaptophysin (Syp), of different lung cell lineages was histologically analyzed in 15 normal human fetal lungs and 12 neuroendocrine tumors (NETs) using immunohistochemical methods. During pseudoglandular phase strong nuclear TTF-1 staining was detected in the columnar nonciliated epithelial cells, while NCAM, CgA and Syp had a moderate expression in the proximal airways and mild expression in the distal airways. Neuroendocrine cells (NECs) in proximal lung airway were co-localizing TTF-1 and other neuroendocrine markers while neuroendocrine bodies (NEBs) exhibit only staining with NCAM and Syp. In the canalicular phase TTF-1 nuclear staining was expressed only in several epithelial cells in proximal airways, while budding airways epithelium showed strong TTF-1 expression. Expression of NCAM, CgA and Syp in this phase equals the one in pseudoglandular phase. NEBs cells were co-localizing TTF-1 and NCAM in proximal airways and few NECs in distal airway were co-localizing TTF-1 and Syp. TTF-1 staining in the saccular phase was limited to subsets of epithelial cells in the proximal airways with stronger positivity in the distal airways. NCAM expression is moderate only in proximal airways, while Syp and CgA show mild expression in proximal and distal airways. NECs were co-localizing TTF-1 and NCAM in proximal lung airway. With regard to NECs, all small cell lung cancer (SCLC) cells had strong TTF-1, NCAM, Syp and CgA positivity and TTF-1 co-localized with other neuroendocrine markers. All pulmonary typical carcinoids were TTF-1 negative, while pulmonary atypical carcinoids were focal positive for TTF-1 and some neoplastic cells co-localized TTF-1 with neuroendocrine markers.

Our results indicate that TTF-1 expression in NECs suggests a possible role in their normal development and differentiation. Our results also indicate that possible cell of origin for poorly differentiated SCLC and some atypical carcinoid could be a progenitor cell in neuroendocrine lineage while in typical carcinoids possible cell of origin is localized in terminally differentiated NECs.

Introduction

Lung cancer is the most common malignancy and a leading cause of cancer deaths worldwide with 5-year survival rate of 15% (Travis et al., 1995). Invasive lung tumors include neuroendocrine tumors (NETs) and non-small lung cancers that account for 20–25% and 75–80% of all lung malignancies, respectively (Bertino et al., 2009; Travis, 2010). According to the World Health Organisation (WHO) classification, NETs are divided into three groups: carcinoid

Abbreviations: AC, atypical carcinoid tumors; CgA, chromogranin A; NCAM, neuron cell adhesion molecule; NEBs, neuroendocrine bodies; NECs, neuroendocrine cells; NETs, neuroendocrine tumors; SCLC, small cell lung cancer; Syp, synaptophysin; TC, typical carcinoid tumors; TTF-1, thyroid transcription factor 1; WHO, World Health Organisation.

* Corresponding author.
E-mail address: vsoljic@gmail.com (V. Soljic).

http://dx.doi.org/10.1016/j.acthis.2015.02.002
0065-1281/

tumors (typical TC/atypical AC), large cell neuroendocrine carcinoma and small cell carcinoma (SCLC) (Travis et al., 2004). NETs exhibit signs of neuroendocrine differentiation and share some common morphologic and immunohistochemical features, as well as molecular characteristics (Valente et al., 2010). Immunohistochemical markers most commonly used to detect neuroendocrine cell differentiation are chromogranin A (CgA), synaptophysin (Syp) and neural cell adhesion molecule (NCAM; CD56) (Addis, 1995; Brambilla et al., 2000; Lyda and Weiss, 2000).

During lung development one of the most intriguing question is how undifferentiated epithelial cells, presented in the early phase, give rise to all specialized cell types in the postnatal lung (Domyan and Sun, 2011; Morrisey and Hogan, 2010; Warburton et al., 2010). The lung epithelium consists of many types of specialized cells that fulfill different functions. Cell types in lung epithelium are divided into basal cells, ciliated cells, Clara cells, neuroendocrine cells (NECs) and type I and type II pneumocytes. It is believed that lung epithelium contains stem cells and progenitor cells that maintain types of differentiated lung epithelium cell population (Hong et al., 2004; Pitt and Ortiz, 2004; Reynolds et al., 2000a). NECs are proposed to be the first specialized cell type during fetal lung development implying that cell proliferation and differentiation within epithelium begins with neuroendocrine progenitor cell (Linnoila, 2006). NECs in the respiratory epithelium are found solitary or in small clusters called neuroepithelial bodies (NEBs) and located usually at branchpoints, while small number of NECs is found in the distal lung airways. NEBs have secretory granules which contain various amines and peptides, CgA, Syp somatostatin, calcitonin, etc. (Van Lommel et al., 1999). NECs regulate pulmonary blood flow, bronchial tonus, immune response and maintain stem cell niche (Domnik and Cutz, 2011). Despite the crucial effects of various amines and peptides secretion, NECs function is still unclear. NEBs are often found at branchpoints near Clara cells. Several studies have shown that after an induced lung injury affecting Clara cells, only Clara cells located near NEBs survive. These findings suggest that NECs maintaining of stem cell niche is necessary for epithelium regeneration (Reynolds et al., 2000a,b,b).

Tumorigenesis and lung development share some similar gene expression pathways. (Cardoso and Lu, 2006; Maeda et al., 2007 Powers and Mu, 2008). Thyroid transcription factor 1 (TTF-1) is a tissue-specific transcription factor essential for the normal development of the lung (Lau et al., 2002b; Maeda et al., 2007) and is a lineage-specific marker for tumors developing from terminal airways (Tanaka et al., 2007). In normal adult lung tissue TTF-1 is expressed in Clara cells and type II pneumocytes (Lau et al., 2002b; Warburton et al., 2010). Recent studies showed that TTF-1 contributes to the pathogenesis of the lung cancer especially to tumor cell differentiation and morphogenesis (Kwei et al., 2008; Weir et al., 2007). TTF-1 expression has been detected in vast lung carcinomas, including NETs (Agoff et al., 2000; Cheuk et al., 2001; Di Loreto et al., 1998; Oliveira et al., 2001; Stenhouse et al., 2004; Sturm et al., 2002). It is successfully used to distinguish metastatic carcinomas of pulmonary origin from others carcinoma (Oliveira et al., 2001; Stenhouse et al., 2004).

Recent studies revealed existence of lung stem cells in adults localized at the bronchioalveolar duct juncture and identified them as a possible cell of origin for non-small lung cancer (Kim et al., 2005). Distinct lung cell populations (stem cells or differentiated cells) can give rise to different types of lung cancer, but also, under the influence of the tumor microenvironment and genetic alterations, different type of lung cancer can arise from the same cell of origin. (Sutherland and Berns, 2010). Although the cell of origin for SCLC is not yet identified it is thought they arise from NECs or NEBs or some undifferentiated neuroendocrine progenitor cells (Park et al., 2011; Sutherland et al., 2011). So far, only one study investigated the co-expression of TTF-1 and neuroendocrine differentiation markers in human lungs during development (La Rosa et al., 2010).

In the present study we investigated spatial and temporal expression and co-expression patterns of TTF-1 and CgA, Syp and NCAM, in two series, respectively, of NETs and human lungs during the different developmental phases in order to possibly clarify the cell of origin of NETs and acquire more insights about pulmonary neuroendocrine system during lung development and tumorigenesis.

Materials and methods

Fifteen normal human fetal lung and 12 NETs were collected from the archives of the Department of Pathology, Cytology and Forensic Medicine, University Hospital in Mostar, Mostar, Bosnia and Herzegovina. Human fetuses were obtained after spontaneous abortions. All fetuses were morphologically normal and without any signs of maceration. Tissue preservation was established by staining every 10th section with hematoxylin and eosin. Fetuses were individually staged by crown-rump length and according to menstrual data. Staging showed that the four fetal lungs were in pseudoglandular phase (12th–16th developmental week), six in canalicular (16th–26th developmental week) and five in saccular phase (26th–36th developmental week) of the lung development. Pulmonary NETs in this study included five SCLC (material obtained after first surgery before chemotherapy or lung biopsies via bronchoscopy), four TCs and three ACs (material obtained after first surgery before chemotherapy). Histological grading of the tumors was performed according to the WHO classification of pulmonary neoplasms (Travis et al., 2004). All tissues were fixed in buffered formalin, routinely embedded in paraffin wax, serially cut (5 μm thick sections) and mounted on glass slides.

The study was approved by the Ethical Committee of the School of Medicine University of Mostar in accordance with the Helsinki Declaration (Williams, 2008).

Immunohistochemistry

Sections were deparaffinized through xylol and processed in graded alcohols and water. Endogenous peroxidase activity was quenched by dipping sections in 3% hydrogen peroxide for 15 min. For antigen retrieval, sections were incubated in citrate buffer pH 9 in microwave oven at 95 °C for 17 min. After cooling to room temperature sections were washed with PBS and then incubated with primary antibodies for one hour: rabbit anti-TTF-1 antibody (diluted at 1:50, LS-C210379, LifeSpan BioSciences, Inc., Seattle, USA), mouse anti-synaptophysin (diluted at 1:30; M0776, DAKO, Gloustrup, Denmark), mouse anti-CD56 (diluted at 1:50; R7251, DAKO, Gloustrup, Denmark), mouse anti-CgA (diluted at 1:3000; M0869, DAKO, Gloustrup, Denmark). After a wash with PBS, sections were incubated in HRP rabbit/mouse system (K5007, DAKO, Gloustrup Denmark) for 30 min. Antigen antibody binding was developed after addition of buffered DAB substrate for 10 min. Sections were washed and counterstained with hematoxylin and mounted for microscope viewing. Sections were examined with Olympus BX51 (Olympus, Tokyo, Japan) microscope. Cells reacting with primary antibody had brown stained nucleus or cytoplasm. For negative control primary antibodies were not included in the staining procedures. Positive control for TTF-1 was thyroid tissue.

Double immunofluorescence staining

Double immunofluorescence staining was performed to examine co-localization and relationship of TTF-1 antibody and neuroendocrine antibodies in the same sections. Sections were

Please cite this article in press as: Miskovic J, et al. Co-expression of TTF-1 and neuroendocrine markers in the human fetal lung and pulmonary neuroendocrine tumors. Acta Histochemica (2015), http://dx.doi.org/10.1016/j.acthis.2015.02.002