Ultrastructural assessment of adenoid cystic carcinoma with emphasis on tumour infiltration periphery

Anna Wegner

Received: 3.02.2009
Accepted: 29.04.2009
Subject: original paper

Head and Neck Surgery and Oncological Laryngology Ward of the Great Poland Cancer Centre in Poznań
Poznań, Poland

Address for correspondence:
Anna Wegner
Head and Neck Surgery and Oncological Laryngology Ward of the Great Poland Cancer Centre in Poznań
Garbary 15 str.
61-131 Poznań, Poland
e-mail: omega35@op.pl

SUMMARY

BACKGROUND: There is a wide variety of morphological and clinical types of tumours of the salivary glands. Almost 30 histological types of these neoplasms are known. Adenoid cystic carcinoma is a rarely occurring malignant epithelial neoplasm. It occurs in major salivary glands,but may also originate in the salivary glands of the respiratory tract.

AIM: The aim of the present study was the ultrastructural assessment of the infiltration periphery of different histological types of adenoid cystic carcinoma of the salivary glands.

MATERIALS AND METHODS: Tissue samples from patients with adenoid cystic carcinoma of the salivary glands were studied. The study group consisted of 30 pts. 21 pts with tumour of parotid, 8 with submandibular and 1 with tumour of sublingual salivary glands. All patients were surgically treated and undergone supplementary treatment using radiotherapy in the Greater Poland Cancer Centre. Assessment of tissue samples was performed using morphological diagnoses and ultrastructural evaluation.

RESULTS: Ultrastructural electron microscopy assessment of adenoid cystic carcinoma revealed differentiation of the tumour cells towards ductal salivary, myoepithelial and pluripotential cells. Epithelial cells showed an increased nucleus-cytoplasm ratio, and their nucleoli were characteristic for actively proliferating cells. The analysis showed histological and structural differences between the central and peripheral parts of the tumour.

CONCLUSIONS: The ultrastructural assessment of adenoid cystic carcinoma revealed that the cells in the peripheral parts of the tumour show a lower degree of maturation than the ones in its centre, peripheral stroma contains fewer collagen fibres, and dominant elements at the periphery of the tumour are proteoglycans and glycosaminoglycans, no histoformative features typical of the principal (central) part of the tumour were found at its periphery.

KEY WORDS: adenoid cystic carcinoma; major salivary glands; ultrastructural studies

INTRODUCTION

There is a wide variety of morphological and clinical types of tumours of the salivary glands. Almost 30 histological types of these neoplasms are known [1, 2, 3, 4]. Malignant tumours of the salivary glands represent 2–4% of head and neck neoplasms. Adenoid cystic carcinoma (*carcinoma adenoides cysticum*) is a rarely occurring malignant epithelial neoplasm. It occurs in major salivary glands (parotid, submandibular, sublingual), but may also originate in the salivary glands of the respiratory tract (*larynx*, *trachea*, *bronchi*) [5, 6].

According to Sikorowa adenoid cystic carcinoma represents 6% of all salivary gland neoplasms [4]. It may occur at any age, but it is more common between the 4th and the 7th decades of life. It develops in both sexes, with slightly higher prevalence in women. It is a slow growing tumour tending to infiltrate surrounding soft tissues, bones and nerves. Typical of this type of neoplasm are distant metastases to lungs, bones and brain, which

can develop many years after primary tumour resection [5, 6, 4, 7]. It also shows a high rate of local recurrence. The treatment of choice is a radical surgery excision with supplementary radiotherapy [8, 9].

Histologically adenoid cystic carcinoma consists of small cells with dark staining nucleus and scant cytoplasm. Tumour cells are arranged around numerous cystic and vesicular spaces in hyalinized stroma. Round, glandular-like spaces are filled with a hyalinized, PAS-positive substance. Within the tumour cribriform structures may neighbour in an alternating manner with solid sheets of cells or with a fine, irregular agglomeration of cells [10, 11, 12, 13].

Seifert described three histological types of this tumour: cribriform, tubular and solid [3]. Usually there are various histological patterns present within a single tumour, and the quantitatively dominant one determines the diagnosis. In the cribriform pattern the small cells with large nuclei are arranged in a concentric manner around cystic-like spaces. Cells that are arranged in singular or several layers forming ductal structures are characteristic for the tubular pattern. The solid pattern contains continuous cellular sheets.

AIM

The aim of the present study was the ultrastructural assessment of the infiltration periphery of different histological types of adenoid cystic carcinoma of the salivary glands.

MATERIAL AND METHODS

Tissue samples from patients with adenoid cystic carcinoma of the salivary glands were studied. The patients were diagnosed and surgically treated in the Otolaryngology Institute of the Poznań University of Medical Sciences. The study group contained 30 subjects. 21 subjects had a tumour of parotid, 8 of submandibular and 1 of sublingual salivary glands. The study group consisted of 18 women and 12 men. The patients' age was from 17 to 87 years. The average age of the patients was 56 years. The average age of women and men was 58 years and 52 years, respectively.

All patients were surgically treated and then subjected to supplementary treatment using radiant energy in the Great Poland Cancer Centre.

During histological examination it was revealed that most of the cancers were pleomorphic, i.e. all histological subtypes were found within one tumour. However, prevailing tissue was taken into consideration during evaluation. Solid type (13 tumours) and cribriform type (11 tumours) occurred the most often. Solid type occurred in 6 cancers.

Assessments were performed using the following procedures:

Morphological assessment, including:
- standard examination of slices after H+E staining,
- immunohistochemical assessment (p53 protein, laminin, fibronectin, D1 cyclin),
- ultrastructural assessment.

In each case the postoperative samples were thoroughly examined histologically. Morphological diagnoses were made by the team of the Biopsy-Diagnostic Laboratory in the Department of Clinical Pathomorphology. Ultrastructural assessment was performed in the Electron Microscopy Laboratory in the Pathomorphology Department of the Poznań University of Medical Sciences.

Materials to be examined using transmission electron microscopy were fixed in Karnovsky's fixative (pH 7.34; temp. 4°C within 24 hours) and subsequently sliced, trimmed and assessed by means of the half-shade technique.

Ultraslices were examined using a Zeiss 900 transmission electron microscope.

RESULTS

Ultrastructural electron microscopy assessment of adenoid cystic carcinoma revealed differentiation of the tumour cells towards ductal salivary, myoepithelial and pluripotential cells. Epithelial cells showed an increased nucleus-cytoplasm ratio, and their nucleoli were characteristic for actively proliferating cells. Typical neoplastic transformation abnormalities of the nuclei were found, including changed staining of nuclei in the presence of basic stain (hyperstaining), abnormal arrangement of hyperchromatin structures, irregular shapes of the nuclei (heteronucleosis) and some polynuclear cells. Ultrastructural examination also showed abnormalities of the cytoplasm: vacuolization, irregular outline of

the cytoplasm, lack of cellular membrane polarization and abnormal cell divisions. Images obtained using electron microscopy showed typical cystic and vesicular spaces responsible for the characteristic cribriform appearance of this neoplasm (Fig. 1). Closer analysis of these spaces showed the presence of a hyalinized PAS-positive substance, which in electron microscopy turned out to be a material containing elements of the basal lamina. The substance contained numerous fibronectin fibres, laminin, type-IV collagen and other basal lamina components.

The main histological features of adenoid cystic carcinoma included pseudocysts, numerous, wide intercellular spaces, abundant basal lamina components and sporadically true glandular lumina. In electron microscopy images groups of cells lying in the stroma containing fibroblasts and collagen fibres of typical ultrastructure were seen. Neoplastic cells differed ultrastructurally from the normal ones. Tumour cells contained large, irregular nuclei, with chromatin clustering under the internal lamina of the nuclear membrane (Fig. 2). Some cells displayed various degrees of degenerative changes, judged by the cytoplasmic changes. In some cells substantial widening of the space between the laminae of the nuclear membrane was observed. This abnormality was concomitant with swelling of rough endoplasmic reticulum vesicles. Polysome depletion and decreased electron density of the cytoplasmic matrix were also seen. Disruption of cellular membranes was accompanied by the presence of cytoplasmic material in the intercellular spaces.

Evaluation of adenoid cystic carcinoma revealed that the tumour cells often did not ad-

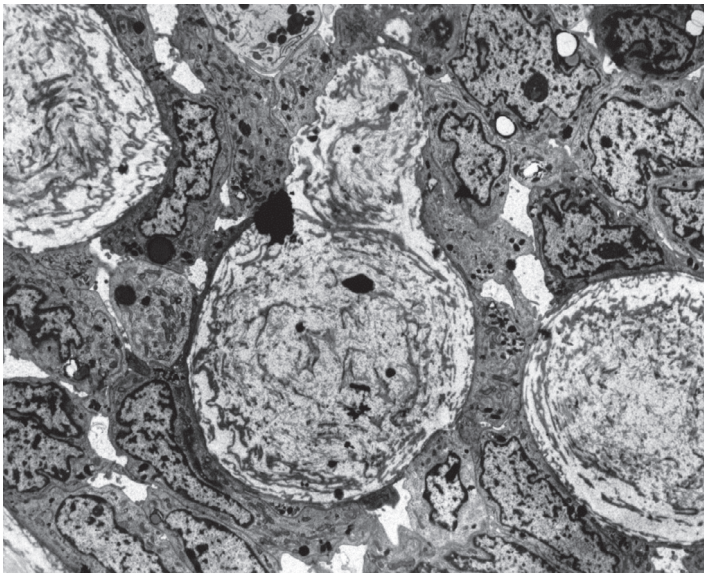

Fig. 1. ACC – Central part of the tumour. Glandular type. Cylindrical spaces surrounded by multiform cells with relatively narrow cytoplasm, with large, polygonal nuclei. In some of the nuclei active nucleoli are to be seen, containing dense chromatin, arranged under the nuclear membrane and in clumps in the euchromatin. The cytoplasm of the cells contains numerous tubular mitochondria and rough endoplasmic reticulum canals and glycogen grains. Some of the cells contain lipid vacuoles. The pseudolumina contain large amounts of a material resembling the elements of the basal lamina and proteoglycan fibres of various sizes. Electron microscopy, magnification 4500x

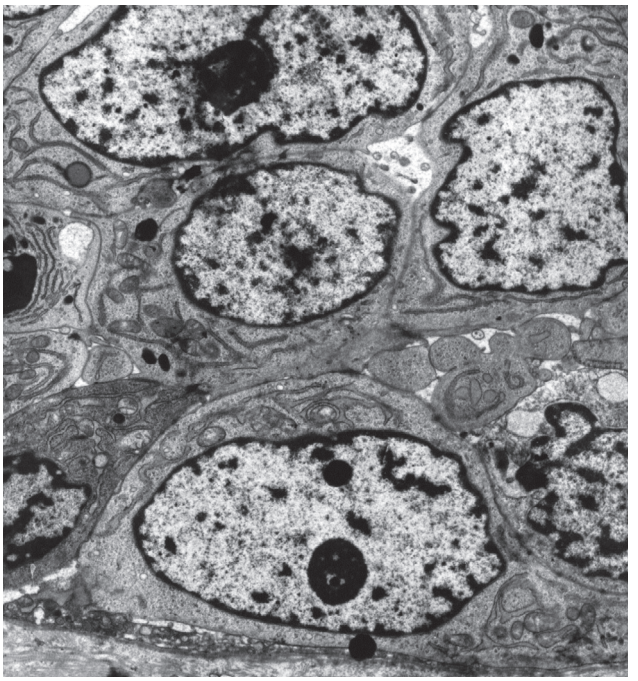

Fig. 2. ACC – Central part of the tumour. Fragment of the solid pattern containing multiform cells with large, irregular nuclei including active nucleoli. Dense chromatin is mostly arranged under the membrane or in clumps. The cytoplasm of the cells is mostly scarce, with relatively numerous canals of rough endoplasmic reticulum, abundant free polyribosomes, not so abundant mitochondria and single lysosomes. The system interconnecting the cells includes desmosome-like links and adhesion-type links. Between the layer of the epithelial cells and the collagenized stroma there is a fragment of the myoepithelial cell cytoplasm and the distinct contour of the basal lamina. Electron microscopy, magnification 11000x

here to each other. Some of them were loosely arranged, with empty spaces between single cells. In some cases these spaces contained amorphous material with cytoplasmic organelles from decayed cells. The cells that were aligned into groups sometimes were attached to each other by numerous desmosome-like links. Some cells formed true glandular lumina – in such cases the "glandular duct" possessed numerous cytoplasmic sprouts protruding into the lumen from the forming cells (Fig. 3).

During the assessment special attention was paid to the periphery of the tumours. The analysis showed histological and structural differences between the central and peripheral parts of the tumour. Histoformative characteristics of the tumour were preserved in its central part – with pseudocysts or ducts and solid cellular areas (Fig. 1, 2, 3). All morpho-

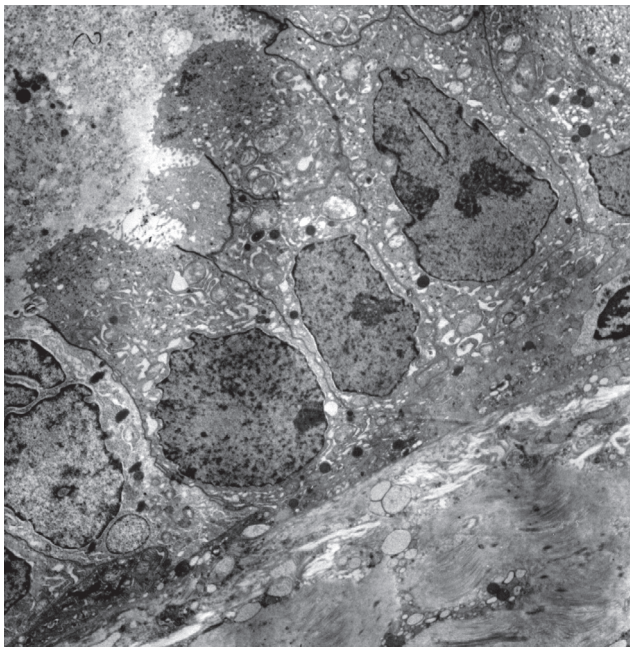

Fig. 3. ACC – Central part of the tumour. Tubular type. Fragment of the tubular pattern consisting of epithelial cells adhering to significantly collagenized stroma, separated from it with basal lamina. Multiform cells building the tubules have numerous sprouts in the form of philopodia or "bridges" connecting the cells with each other. The glandular pole of the cells is made of cellular membrane with microvillar tips. The cytoplasm contains relatively numerous canals of rough endoplasmic reticulum, mitochondria, free polyribosomes, singular lysosomes and abundant fibrillae in the cytosol, with differing orientation in relation to the nuclei. In the lumen of the tubules there can be found an amorphous, electron-dense material. Electron microscopy, magnification 5600x

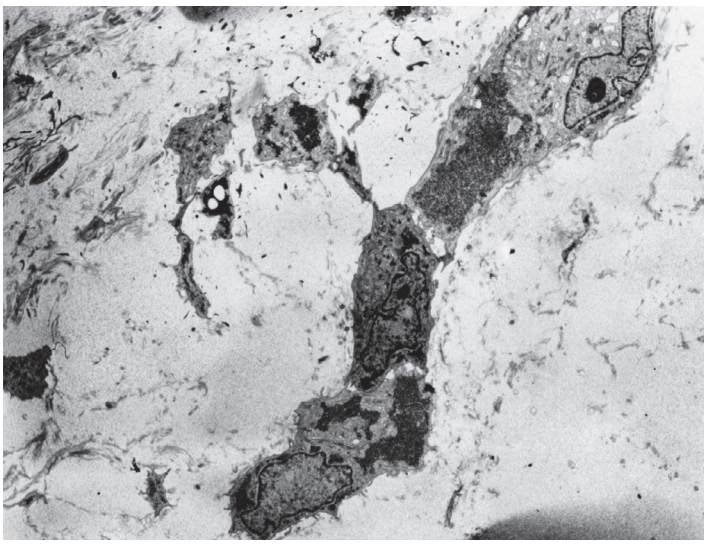

Fig. 4. ACC. Peripheral part of the glandular-cystic cancer, solid type. The complex of 3 cells "floats" in the loose stroma of the intercellular space, containing glycosaminoglycans (GAG), singular collagen fibres, fine cytoplasmic sprouts of other cells. Magnification 5600x

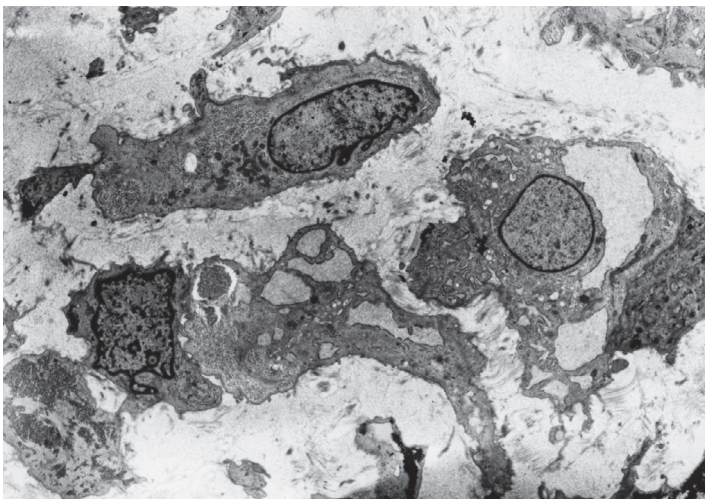

Fig. 5. Complex of cells from the peripheral part of the tumour. Two of the visible cells contain canals of rough endoplasmic reticulum with fine fibrillic material resembling the material of glycosaminoglycans. One of these cells and one not mentioned before show portions of cytoplasm containing glycogen grains. Apart from glycosaminoglycans (GAG) the intercellular space contains groups of collagen fibres, which focally arrange parallel to each other. No tendency toward bundle formation was observed. Magnification 8750x

logical forms were accompanied by distinct stromal structures – collagen, proteoglycans, glycosaminoglycans and sporadic elastin fragments. In peripheral parts of the tumour the cells formed scarce groups, with short cellular cords and singular cells (Fig. 4, 5). No ductal forms or pseudocyst formations were observed. In most peripheral parts only single cells were seen. Most cellular groups showed extensive decrements or lack of basal lamina. Quite often the cells that became separated from the main group showed signs of complete depolarization and floated in a loose stroma with scarce fibrillar elements (Fig. 6). Glycosaminoglycans were the main feature of the stroma, with less abundant collagen fibres. Fully expressed depolarization of the cells in the peripheral parts of the tumour was the principal feature that differentiated it from the central parts. The nuclei of these cells were morphologically similar to those of the cells from the central parts. Moving towards the centre of the tumour revealed increasingly pronounced features of adhesion of the elements of the tumour. In the peripheral parts a reverse trend was seen – the further towards the periphery, the looser were the connections, reaching the point where there was a total lack of links between the elements of the tumour.

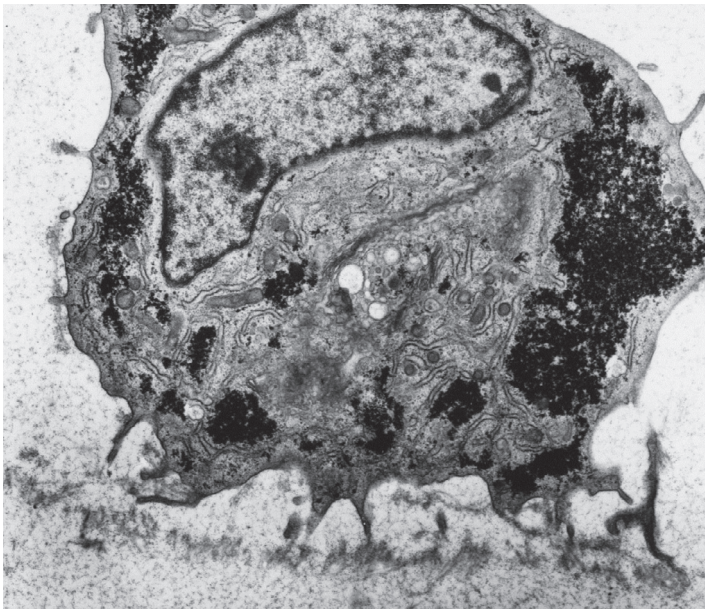

Fig. 6. ACC – peripheral part of the tumour. Cancer cell "floating" in the intercellular space, some of its cytoplasmic sprouts reach collagen fibres surrounded by glycosaminoglycans (GAG). The structure of the cytoplasm does not differ from that of the solid pattern cells. Relatively large proportion of the cytoplasm filled with glycogen grains. Magnification 24500x

DISCUSSION

Adenoid cystic carcinoma (ACC) differs from other salivary gland cancers in the clinical and histological picture and in the unpredictable course of the disease. Histological, ultrastructural and genetic studies, as well as molecular techniques, progress in which dominated in the last decade of the 20th century, are used in order to improve knowledge of the biology of this cancer.

Conducted ultrastructural studies of ACC revealed typical anomalies of nucleus and cells which are characteristic for cancer transformation and confirmed the main features of histoarchitecture of ACC.

Also, electron microscope studies of the cancer allow location and layout of the extracellular matrix components to be assessed.

In our studies we maintained the presence of laminin and fibronectin inside the lumen of pseudocysts and in the extracellular matrix. D'Ardenne, who assessed 7 cases of ACC, and Dong, who examined 22 cases of cancer using a transmission electron microscope, obtained similar results.

In histological and immunocytochemical diagnostics, staining and localization of the basement membrane components, such as laminin and type IV collagen, may be of great importance in the differentiation between invasive and non-invasive cancer.

Hua affirmed greater amounts of laminin in high-metastatic clones of ACC and observed an increase in cell migration rate in comparison with low-metastatic clones. It seems that this could be of significant importance for ACC prognostication.

During electron microscopic examination special attention was paid to the evaluation of infiltration of periphery and its comparison with the tumour centre. On the periphery there were found less mature cells and lack of histoarchitecture which is typical for the centre. In the available literature, there are few reports on electron microscopic studies of ACC of salivary glands. No studies focused on careful evaluation of

the tumour periphery have been conducted. This area may bring new, significant information about ACC of salivary gland prognostication.

CONCLUSIONS

The ultrastructural assessment of adenoid cystic carcinoma revealed that:

The cells in the peripheral parts of the tumour show a lower degree of maturation than the ones in its centre.

Peripheral stroma contains fewer collagen fibres, and dominant elements at the periphery of the tumour are proteoglycans and glycosaminoglycans.

No histoformative features typical of the principal (central) part of the tumour were found at its periphery.

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