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Differential proteomic and phenotypic behaviour of papillary and anaplastic thyroid cell lines☆

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ABSTRACT

Thyroid carcinomas account for a minority of all malignant tumours but, after those of the gonads, they represent the most common forms of endocrine cancers. They include several types, among which the *papillary thyroid cancer* (PTC) and the *anaplastic thyroid cancer* (ATC) are the best known. The two hystotypes display significant biological and clinical differences: PTC is a well differentiated form of tumour with a high incidence and a good prognosis, while the ATC is less frequent but represents one of the most aggressive endocrine tumours with morphological features of an undifferentiated type. To date, as far as we know, no conclusive studies, useful to design arrays of molecular markers, have been published illustrating the phenotypic and proteomic differences between these two tumours. The aim of this work was to perform a comparative analysis of two thyroid cancer cell lines, derived respectively from papillary (BCPAP) and anaplastic (8505C) thyroid carcinomas. The comparative analysis included cell behaviour assays and proteomic analysis by 2D-PAGE and mass spectrometry. The results have highlighted a new proteomic signature for the anaplastic carcinoma-derived cells, consistent with their high proliferation rate, motility propensity and metabolic shift, in relation to the well-differentiated PTC cells.

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1. Introduction

The human thyroid gland is composed of a basic structural unit, the *follicle*, consisting of a monolayer of well polarized cells, the *thyrocytes*, responsible for the T3/T4 hormone secretion, and of other peripheral cells, the *parafollicular C cells*, responsible for the secretion of calcitonin. The presence within the follicle of stem cells, or remnants of embryonic cells, has been hypothesized as the target cells for tumour initiation. A thin extracellular matrix, which includes occasional fibroblasts and inflammatory cells, is peripheral to the follicle structure.

Thyroid carcinomas account for 1–2% of all malignant tumours and, after those of the gonads, they represent the most common tumours of the endocrine system. The thyroid

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tumours include several histotypes with different molecular profiles, as well as biological and clinical behaviours.

Among these, papillary (PTC) and anaplastic (ATC) histotypes are probably the most investigated. The two hystotypes display significant phenotypic differences, as well as dissimilar clinical occurrences and outcomes. Indeed, PTC is a well differentiated form of tumour with a high incidence, representing approximately 80% of all thyroid tumours and is characterized by a good prognosis [1]. On the contrary, ATC accounts for less than 5%, but it is one of the most aggressive endocrine tumours with morphological features of an undifferentiated type.

However, to date no studies have definitely demonstrated whether malignant forms of thyroid tumours arise from the adult epithelial cells through multistep cancerogenesis [2] or from remnants of foetal thyroid cells [3] or, as more recently hypothesized, from resident stem cells [4–6].

Many genetic alterations have been described as involved in the progression, mostly leading to the anomalous activation of the MAP kinase pathway. Several studies have suggested that $BRAF^{(V600E)}$ mutation (90% of all BRAF mutations) plays an important role in the early steps of the thyroid carcinogenesis leading to the progression towards the anaplastic forms [7–10], but some controversies about its significance still remain.

Therefore, the biological mechanisms of thyroid cancerogenesis are still unclear. This is also because the panels of putative biomarkers for thyroid cancer histotypes are not yet adequate enough to fulfil the requirements for molecular diagnosis, prognosis and target therapy. We suggest that the increase in knowledge of protein expression in thyroid tumours, as for the breast cancer [11–13], could greatly help to understand molecular mechanisms involved in the thyroid carcinogenesis. With this aim, we performed a comparative analysis of two thyroid cancer cell lines, derived respectively from papillary (BCPAP) and anaplastic (8505C) thyroid carcinomas. The comparative analysis included cell behaviour assays, proteomic analysis by 2D-PAGE and mass spectrometry.

First of all, in this study we show that both PTC and ATC cell lines closely maintain *in vitro* phenotypical characteristics, probably resembling their *in vivo* counterparts. Indeed, the 8505C cells are characterized by cell traits and a behaviour typical of the aggressive phenotype associated with an advanced stage of the disease. On the contrary, the BCPAP cells derived from a more differentiated tumour, maintain *in vitro* a rather stable phenotype and the ability to reorganize "follicle-like" structures. Our findings confirm that these cell lines represent an important model for the *in vitro* study of differentiated and undifferentiated thyroid tumours and may offer new insights into the thyroid carcinogenesis. The proteomic study revealed a panel of differentially expressed proteins, instrumental for cancer growth and invasion, which may be used for future applications as biomarkers of thyroid malignancy.

2. Materials and methods

2.1. Cell culture

The human papillary thyroid carcinoma cell line, BCPAP established in 1992 [14] and human anaplastic thyroid carcinoma cell line, 8505C established in 1994 [15] were provided by the Endocrinolgy Labolatory of Prof. C. Giordano. Cells were seeded at a density of 10^4 cells/cm^2 and grown in RPMI 1640 (GIBCO), supplemented with 10% foetal bovine serum (GIBCO), 1% L-glutamine, 1% penicillin and 1% streptomycin in a humidified incubator with 5% CO_2 in air at 37 °C.

2.1.1. Cell proliferation

The assay [16] was performed by the use of a colorimetric tetrazolium compound (CellTiter 96, Promega). Briefly, 20 μl of CellTiter 96 was added to 100 μl of medium into each well containing the cells. After 1 h of incubation in a humidified 5% CO_2 atmosphere, the absorbance at 492 nm was read using a 96-well plate reader (Amersham).

2.1.2. Scratch assay

The confluent mono-layers of BCPAP and 8505C cells were scraped with a p200 pipet tip. Following the "scratch", the cells debris were removed by several washes with phosphate-buffered saline (PBS) and fresh growth medium was added. The assays were monitored at different times (6 h–24 h) through optical microscopy.

2.2. Gelatin zymography

2.2.1. Conditioned media preparations

Conditioned media of BCPAP and 8505C confluent cells were collected after 24 h of culture in absence of serum. The media were then submitted to extensive dialysis against ultrapure distilled water at 4 °C and lyophilized. Dried samples were solubilized in a buffer 50 mM Tris–HCl, pH 7.5.

2.2.2. Zymograpic assay

Aliquots corresponding to 10 μg were used for mono-dimensional gelatin zymography, that was performed under non-reducing conditions, on 7.5% SDS-PAGE copolymerized with 0.1% gelatin, using a minigel lab apparatus (Biorad). Following the electrophoresis, the SDS was removed from the gel by washes of 1 h with 2.5% Triton-X 100 in 50 mM Tris–HCl, pH 7.5. The zymogram was subsequently developed for 18 h at 37 °C in a buffer containing 50 mM Tris–HCl, pH 7.5, 0.15 M NaCl and 10 mM $CaCl_2$ [17]. Gel was stained with Coomassie blue and unstained areas corresponding to zones of digestion were visualized after destaining with 7% methanol in 5% acetic acid.

2.2.3. Western blotting

Zymographic controls were performed by western blot assay with purified samples of proMMP-2 and proMMP-9 (kindly donated by Dr H. Nagase, Imperial College, UK), probed respectively with one of the two monoclonal antibody: anti-MMP-2 mouse mAb (1:1000; Santa Cruz, Heidelberg, Germany) and anti-MMP-9 mouse mAb (1:1000; Santa Cruz). Following incubation with the appropriate peroxidase-linked antibody [horseradish peroxidase-conjugated goat anti-mouse IgG (1:3000; Santa Cruz)], the reaction was revealed by the ECL detection system, using high performance films (Hyperfilm ECL; Amersham).

2.3. Scanning electron microscopy (SEM)

BCPAP and 8505C cells were seeded in T-25 cell culture flasks at a concentration of 15×10^3 cell/cm^2 and were processed for

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