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PROX1 TRANSCRIPTION FACTOR IS DIFFERENTIALLY EXPRESSED IN THYROID CANCER AND CONTRIBUTES TO THE REGULATION OF INVASION AND MIGRATION OF THYROID CANCER CELLS

Rudzińska M.[1], Karpińska K.[1], Gaweł D.[1], Kiedrowski M.[2], Stępień T.[3], Marchlewska M.[3], Krasuska W.[1], Ledwoń A.[1] , Macios M.[1], Czarnocka B.[1]*

1. Department of Biochemistry and Molecular Biology, Center of Postgraduate Medical Education, Warsaw 2. Department of Pathology, Maria Skłodowska-Curie Memorial Cancer and Institute of Oncology, Warsaw 3. Department of General and Endocrinological Surgery, Copernicus Memorial Hospital, Łódź

INTRODUCTION

The dissemination of differentiated thyroid cancers varies. It is believed that papillary thyroid carcinomas (PTCs) metastasize by lymphatic spread, whereas follicular thyroid carcinomas (FTCs) generally via hematogenous spread. The molecular mechanisms of these processes are still unclear. One of the key genes involved in lymphangiogenesis is prospero-homeobox 1 (*PROX1*). Prox1 is a transcription factor important in the regulation and maintenance of the lymphatic endothelial phenotype. Prox1 is critical for organs development during embryogenesis and it was also described as a tumor-suppressor gene. **The aim** of our study was to determine the potential role of Prox1 in the regulating of the hallmarks of malignant cell phenotype (cellular migration, invasion and anchorage independent growth) in several thyroid cancer cell lines.

METHODS

Studies were performed on a series of thyroid cancer cell lines derived from human papillary (TPC1 and BcPAP) and follicular (FTC-133 and CGTH-W-1) thyroid carcinomas. Nthy-ori 3-1 cell line (a normal human thyroid cells) was used as a control. The protein and transcript expression levels, cellular localization of Prox1 and cytoskeleton changes were determined using: Q-RT-PCR, Western Blot, IF methods. The Prox1 expression was examined also in a series of archived thyroid cancer and normal thyroid tissues by IHC. The effect of Prox1 on cell invasiveness and colony formation was investigated using FTC-133 cell line after transfection with Prox1 siRNA (gene expression silencing) and plasmid containing GFP-tagged *PROX1* construct (overexpression).

RESULTS

PROX1 EXPESSION IN ESTABLISHED CELL LINES AND TISSUES

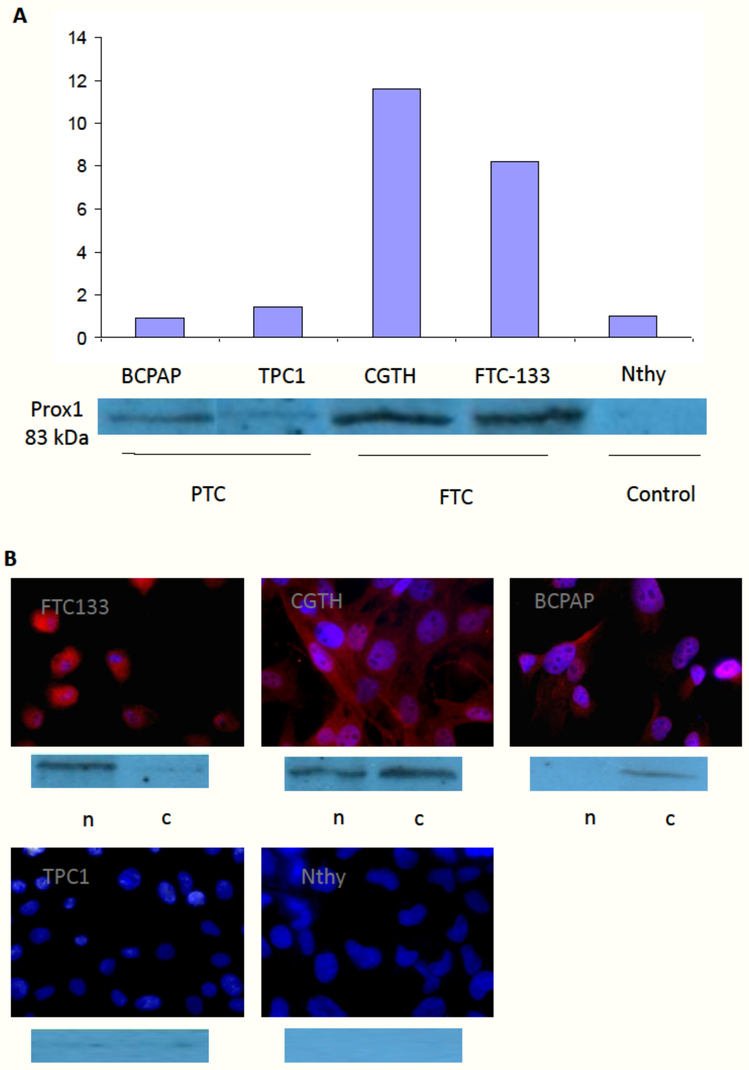

Fig.1. Prox1 expression in human thyroid cancer cell lines and tissues: **A**. mRNA and protein levels **B**. subcellular localization (n, nuclear; c, cytoplasmic) **C**.IHC staining a) normal thyroid tissue, b-d) follicular thyroid carcinomas.

PROX1 SILENCING

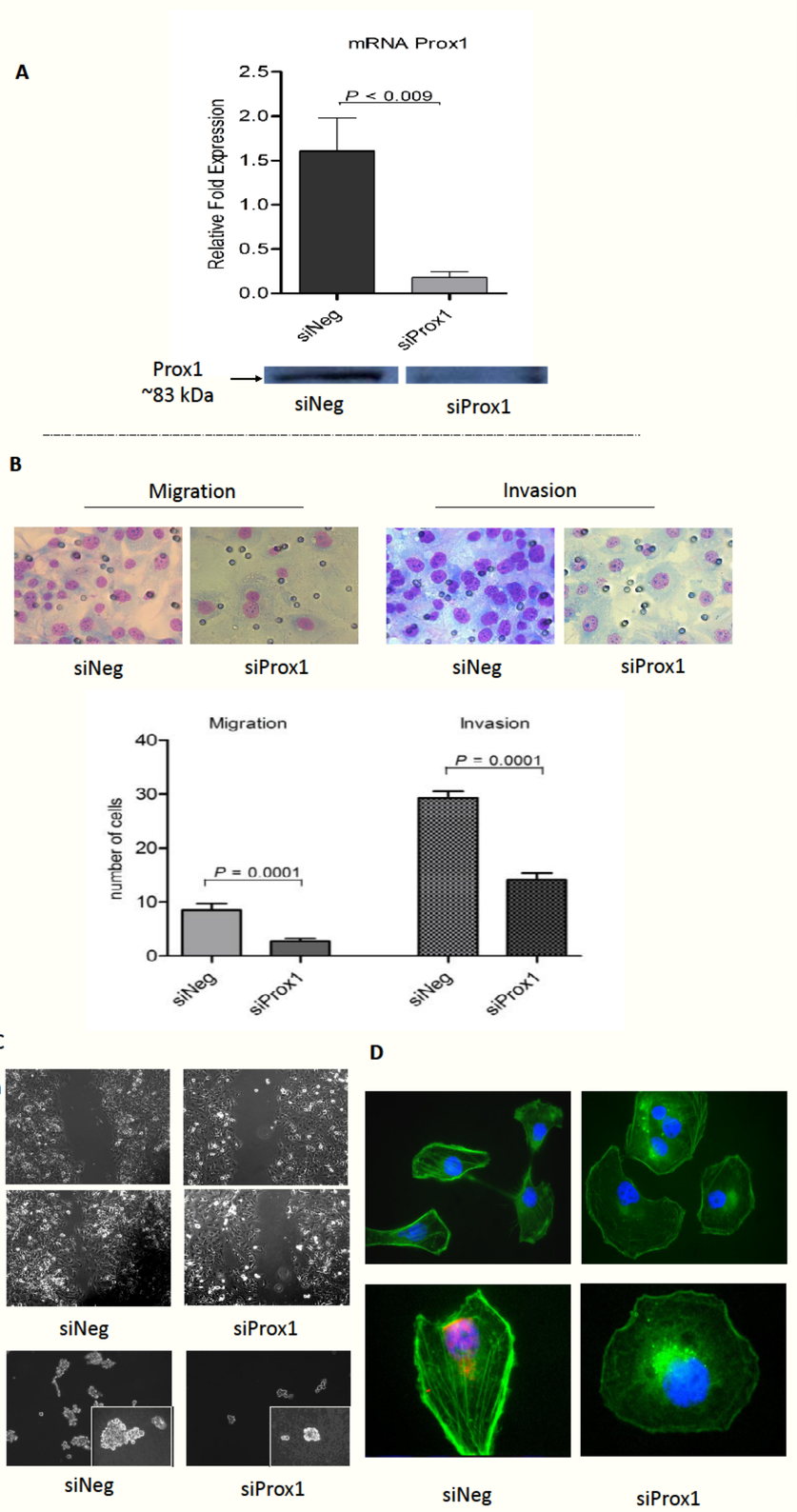

Fig.2. Prox1 is involved in the regulation of cancer progression: **A**. Prox1 knock-down with siRNA in FTC-133 cell line. **B.** Representative images of migration and invasion of FTC-133 cells after Prox1 silencing. **C.** Motility (scratch assay) and anchorage-independent growth (colony formation assay) of FTC-133 cells upon Prox1 knock-down.**D**. Cytoskeleton staining.

PROX1 OVEREXPRESSION

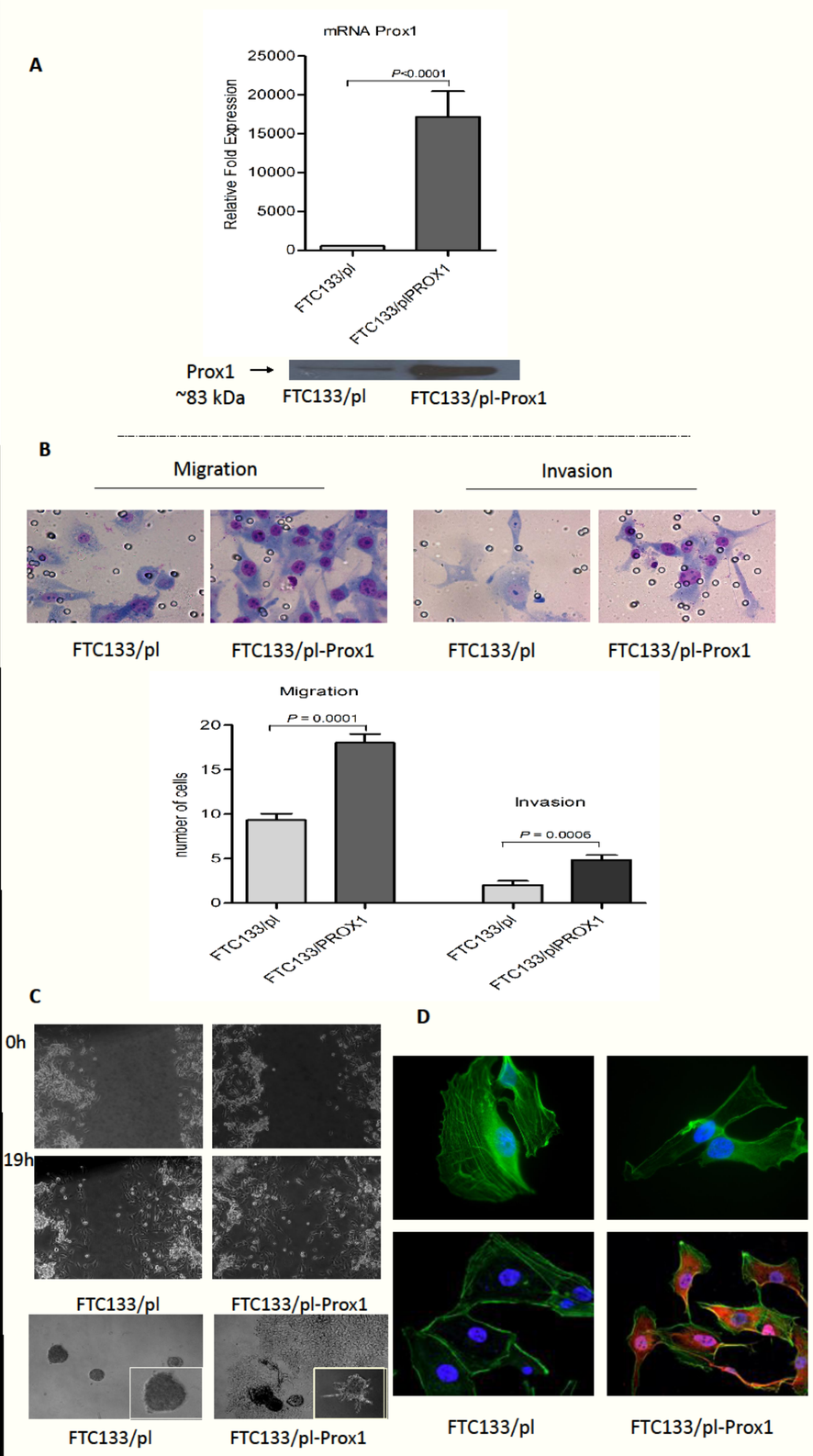

Fig.3. **A**. Prox1 overexpession in FTC-133 cell line. **B.** Representative images of migration and invasion of FTC-133 cells after overexpession of Prox1 **C. M**otility (scratch assay) and anchorage-independent growth (colony formation assay in Matrigel) of FTC-133 cells after Prox1 overexpession.**D**. Cytoskeleton staining.

Prox1 expression considerably differs between FTC and PTC derived cell lines. High Prox1 mRNA and protein levels were seen in FTC-133 and CGTH cells, whereas in cell lines originating from PTC low (BcPAP) or undetectable (TPC1) levels were present (Fig.1A). Protein was localized in the nucleus (FTC-133) or in the nucleus and cytoplasmic compartment (CGTH and BcPAP), respectively (Fig.1B). IHC analysis revealed its expression in FTCs with modest to intensive staining localized in both cytoplasm and nucleus, and not in the normal thyroid tissue (Fig.1C). Prox1 is involved in the regulation of features of cancer progression. Prox1 silencing (Fig.2A) reduced migration and invasive potential of FTC-133 cells (Fig.2B), as well as the motility and anchorage-independent growth (Fig.2C), whereas its overexpression in FTC-133 cells (Fig.3A) promoted the invasiveness (Fig.3B) and anchorage-independent growth (Fig.3C). Prox1 silencing or overexpression deeply influenced FTC-133 cells morphology through the rearrangement of the actin cytoskeleton (Fig.2D, 3D).

CONCLUDING REMARKS

Prox1 is highly expressed in FTC cell lines, while in PTC derived cell lines it is downregulated. Prox1 is involved in the regulation of key hallmarks of tumor progression. Knock down of Prox1 expression in FTC-133 cells reduces their migration, invasiveness and anchorage independent growth, whereas its overexpression in FTC-133 cells promotes migration and invasive potential. Changes in Prox1 expression substantially altered cells morphology influecing FTC-133 cells invasive potential. These observations suggest that Prox1 may play important role in the regulation of malignant cell phenotype.

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