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Nongenomic activation of phosphatidylinositol 3-kinase signaling by thyroid hormone receptors

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ABSTRACT

Thyroid hormone (T3) is critical in growth, development, differentiation, and maintenance of metabolic homeostasis. Recent studies suggest that thyroid hormone receptors (TRs) not only mediate the biological activities of T3 via nucleus-initiated transcription, but also could act via nongenomic pathways. The striking phenotype of thyroid cancer exhibited by a knockin mutant mouse that harbors a dominant negative TRβ mutant ($TR\beta^{PV/PV}$ mouse) allows the elucidation of novel oncogenic activity of a TRβ mutant (PV) via extra-nuclear actions. PV physically interacts with the regulatory p85α subunit of phosphatidylinositol 3-kinase (PI3K) to activate the downstream AKT-mammalian target of rapamycin (mTOR) and $p70^{S6K}$ and PI3K-integrin-linked kinase-matrix metalloproteinase-2 signaling pathways. The PV-mediated PI3K activation results in increased cell proliferation, motility, migration, and metastasis. Remarkably, a nuclear receptor corepressor (NCoR) was found to regulate the PV-activated PI3K signaling by competing with PV for binding to the C-terminal SH2 domain of p85α. Over-expression of NCoR in thyroid tumor cells of $TR\beta^{PV/PV}$ mice reduces AKT-mTOR-$p70^{S6K}$ signaling. Conversely, lowering cellular NCoR by siRNA knockdown in tumor cells leads to over-activated PI3K-AKT signaling to increase cell proliferation and motility. Furthermore, NCoR protein levels are significantly lower in thyroid tumor cells than in wild type thyrocytes, allowing more effective binding of PV to p85α to activate PI3K signaling, thereby contributing to tumor progression. Thus, PV, an apo-TRβ, could act via direct protein–protein interaction to mediate critical oncogenic actions. These studies also uncovered a novel extra-nuclear role of NCoR in modulating the nongenomic actions of a mutated TRβ in controlling thyroid carcinogenesis.

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1. Introduction

Thyroid hormone (T3) has diverse effects on growth, development, differentiation, and maintenance of metabolic homeostasis. Thyroid hormone nuclear receptors (TRs) mediate these biological activities via transcriptional regulation. TRs are derived from two genes, α and β, that are located on two different chromosomes. Alternate splicing of primary transcripts gives rise to four T3-binding TR isoforms: α1, β1, β2, and β3. The expression of these TR isoforms is developmentally regulated and tissue-dependent [1]. TRs regulate transcription by binding to the thyroid hormone response elements (TREs) in the promoter regions of T3-target genes [1]. In addition to the effects of T3 and the various types of

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TREs, transcription activity of TR is modulated by tissue- and development-dependent TR isoform expression [2,3] and by a host of corepressors and coactivators [3,4]. Studies using TR subtype knockout and TR total knockout mice have shown that TR isoforms have subtype-specific and redundant functions [5].

Recent studies, however, have indicated that TRs could also mediate T3 biological activities beyond TRE-mediated gene transcription. Simoncini et al. first reported T3-dependent TR-mediated activation of phosphatidylinositol 3-kinase (PI3K) activity in human endothelial cells [6]. This activation is through direct physical interaction of TR with the p85α subunit of PI3K, leading to the phosphorylation and activation of AKT and endothelial nitric oxidase synthase [7]. This TR-mediated PI3K activation has also been demonstrated in other cell types including human fibroblasts, neonatal rat cardiomyocytes, and human and rat insulinoma cell lines [8–12]. These studies indicate that non-TRE-dependent effects of TR may contribute to important physiological effects of T3.

The unliganded TRs (apo-TRs) play critical roles that is evident in the congenital hypothyroidism leading to cretinism with growth defects and mental retardation [13]. Studies of mice deficient in all TRs ($TR\alpha1^{-/-}$ and $TR\beta^{-/-}$ mice) have shown that they exhibit a milder overall phenotype than the debilitating symptoms of severe hypothyroidism [5,14], highlighting the important role of apo-TRs in the pathogenesis of hypothyroidism. Current interest in nongenomic actions of TRs has focused more on the liganded state. Thus, whether apo-TRs could contribute to diseases via nongenomic actions is less well understood. The availability of a knockin mouse harboring a dominant negative TRβ mutation ($TR\beta^{PV/PV}$ mouse) presents an opportunity to address this question. The PV mutation was identified in a patient with resistance to thyroid hormone (RTH). It is due to a C-insertion at codon 448 of the TRβ1 that leads to a mutant that has completely lost T3 binding and transcription activity [15,16]. The TRβPV mouse faithfully reproduces human RTH with dysregulation of the pituitary–thyroid axis [17]. Remarkably, as $TR\beta^{PV/PV}$ mice age, they spontaneously develop follicular thyroid carcinoma similar to human thyroid cancer [18,19]. We therefore used this mouse model to ascertain whether apo-TRβ could also signal via the PI3K pathway to mediate its oncogenic actions. This article will highlight recent advances in the understanding of the molecular mechanisms of the nongenomic actions of apo-TRs *in vivo* achieved with the use of a mouse model of thyroid cancer ($TR\beta^{PV/PV}$ mouse).

2. Activation of PI3K by PV via nongenomic pathways

2.1. *Potent activation of PI3K by PV via protein–protein interaction*

Amplification of the PIK3C gene and activation of PI3KC are frequently observed in human follicular thyroid cancer [20]. $TR\beta^{PV/PV}$ mice that spontaneously develop follicular thyroid cancer provide a valuable tool to investigate whether an apo-TRβ (PV) could activate PI3K signaling via nongenomic pathways. Analysis of PI3K activity in the thyroid extracts indicates that those of wild type mice show weak PI3K activity. The PI3K activity in the thyroid of $TR\beta^{PV/PV}$ mice is significantly higher than in wild type mice (40–50-fold) [21].

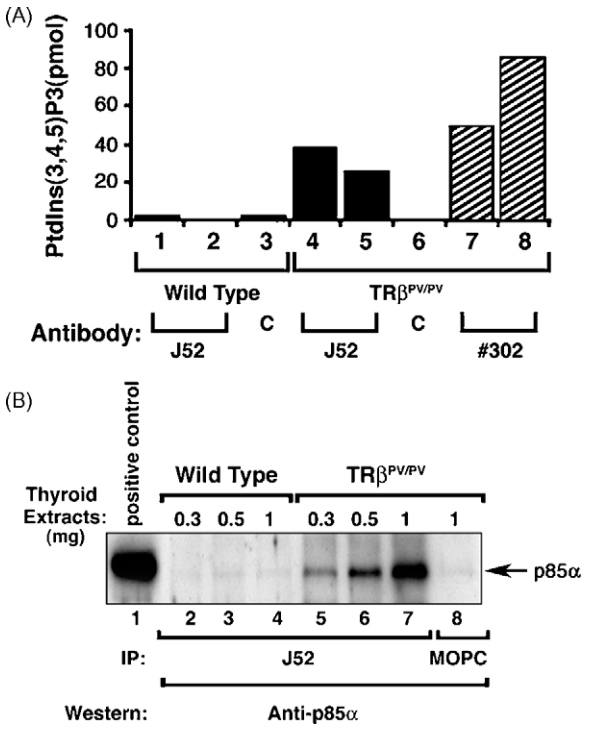

Fig. 1 – Physical interaction of p85α with TRβ or PV in the thyroid extracts of wild type and $TR\beta^{PV/PV}$ mice, respectively. (A) The PI3K activity is associated with TRβ or PV. One hundred micrograms of proteins derived from the total thyroid extracts of wild type (bars 1–3; three mice) or $TR\beta^{PV/PV}$ mice (bars 4–8) were immunoprecipitated with 5 μg of anti-TRβ1 (J52, bars 1 and 2, wild type mice; bars 4 and 5, two $TR\beta^{PV/PV}$ mice), anti-PV (#302; bars 7 and 8, two mice) antibodies, or an irrelevant monoclonal antibody (MOPC) as control (bars 3 and 6, marked as C), and the PI3K activity was determined. (B) Co-immunoprecipitation of p85α with TRβ or PV. Increasing concentrations of lysates from pooled thyroid extracts of six wild type mice or three $TR\beta^{PV/PV}$ mice, respectively, were immunoprecipitated with J52 antibody and subjected to immunoblot analysis probed with anti-p85α antibody. Lane 1 is Jurkat cell lysate (#12-303, Up-State) as a positive control.

PI3K associates with TRs in human vascular endothelial cells and fibroblasts [7,8], but whether PV, an apo-TRβ, is associated with PI3K in the thyroid was unknown. We therefore used monoclonal antibody J52 [22], which recognizes the N-terminal region of the A/B domain of TRβ and PV, to determine whether these two TRs are associated with PI3K. Fig. 1A shows that the antibody J52 precipitated from thyroid extracts of $TR\beta^{PV/PV}$ mice (Fig. 1A, bars 4 and 5) had 30-fold more PI3K activity than wild-type mice (bars 1 and 2). The increased PV-associated PI3K activity is not due to preferential binding of J52 with PV, because J52 interacts with TRβ and PV with a similar affinity as J52 recognizes the epitope in the A/B domain shared by TRβ and PV. To be certain that the increased PI3K