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Short Communication

T3 Directly Regulates Epithelial Morphogenesis via E-Cadherin-Catenins-Rac1

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Keywords

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Abstract

Recent findings of our group show strong evidences that T3 is a direct regulator of gastrointestinal epithelial morphogenesis via E-cadherin-catenins-Rac1 pathway. Thus, T3 differentially mediate cell proliferation and differentiation promoting the configuration and functionality of the epithelial barrier. By using gene expression, bioinformatics and morphometric tools we have demonstrated this physiological signaling route. Our results open therapeutic possibilities not only for thyroid disorders management, but also for pathological imbalances between cell proliferation-differentiation events that occur in diseases such as colon cancer.

ABBREVIATIONS

T3: 3,5,3'-Triiodothyronine; TH: Thyroid Hormone; Rlp8: Ribosomal Protein L8; G: Larval Stage of Gosner 1960; NF: Nieuwkoop and Faber Larval Stages; RTβ: Hormone Receptor Beta; IFABP: Intestinal Fatty Acid Binding Protein; TRE: Thyroid Hormone Response Element; PWM: Position Weight Matrices; Tfs: Transcription Factors; AJC: Apical Junctional Complex.

INTRODUCTION

Postembryonic animal remodeling by thyroid hormones regulation is an ancestral feature of chordates, and anuran metamorphosis is an extreme example of a widespread life history transition [1]. This developmental period is fully open to environment changes as well as genetic (and epigenetic) factors, in which thyroid hormones play a central role. Therefore, the study of anuran gastrointestinal metamorphosis is a key model to better understand its physiology and pathology in mammals [2,3], including humans.

Amphibian-digestive tract morphogenesis results from tissue disruption and larval cell apoptosis, stem cell proliferation and differentiation to replace larval epithelial, connective and muscle tissues. These events involve a deep disassembly-reassembly process of cell-cell and cell-extracellular matrix contacts [4,5]. Between these adhesive contacts, the cadhesomes [6] have critical roles in epithelial morphogenesis. These molecular complexes act integrating signals from extracellular and intracellular environments [5,7,8].

Our results and those reported by other authors allow to deepen the knowledge of the molecular mechanisms involved in the dynamic regulation of gastrointestinal epithelial cell-cell adhesive contacts in physiological and pathological stages [2,5,9].

MATERIALS AND METHODS

Spontaneous and 3,5,3'-Triiodothyronine (T3)-induced metamorphosis

Rhinellaarenarum (stage G33, [10]) and *Xenopus laevis* (stage NF53, [11]) pre-metamorphic tadpoles were maintained in artificial pond water at a population density of 10 larvae/l at 20 ± 2°C under a 12:12 h light-dark photoperiod up to final metamorphosis (G45 and NF66, respectively).

Pre-metamorphic tadpoles G33 and NF53 were immersed in 1.25 nM and 7.5 nM T3-solutions respectively (Sigma-Aldrich Co. St. Louis, MO, USA) for 24 hours (T324h) and 5 days (T35d), and T3-effects compared with those in control groups pre- and post-metamorphic stages (G33, NF53 and G45, NF 66). Solutions were daily renewed and animals maintained without feeding. Bioassays were performed by quintuplicate, according to preliminary assayed conditions and previous reports [4,12,13].

RT-PCR Gene expression quantification

Digestive tracts of *X. laevis* were dissected and immediately used for total-RNA extraction. Nine digestive tracts were pooled for each of five replicates per treatment. First strand cDNAs were obtained by retro-transcription (Thermo Scientific Inc., Maryland, USA). The PCR reactions were developed from cDNAs and gene-specific primers. The mRNAs levels were established

by co-amplification of each interest gene with the ribosomal protein L8 (rlp8) constitutive gene [14], from larval digestive tracts of NF53, NF66, $T3_{24h}$ and $T3_{5d}$, and visualized in ethidium bromide-stained 2.5% agarose gel. The expression levels were quantified by Image J software and band-intensities ratios were calculated. The control group value was considered as 1, whereas the treatment-group values were analyzed regarding to control. The difference between means was evaluated according to Fay and Gerow [15]. The thyroid hormone receptor beta (RTβ) mRNA quantification was used as positive control of a T3-direct response gene, whereas the intestinal fatty acid binding protein (IFABP) mRNA was used as a positive control of T3 inhibitory-response gene [16].

Bioinformatics' studies

Genes involved in cadhesome organization and dynamics were analyzed to predict *cis* regulatory sequences, particularly T3 response elements (TREs). The DNA binding affinity of transcription factors (TFs) was analyzed by means of the Position Weight Matrices method (PWM) [17] by using the INSECT2.0 tool [18].

Morphological and morphometric studies

Control and T3-treated tadpoles were fixed and their digestive tracts were dissected, processed and analyzed by wild field and electron transmission microscopies, according to Izaguirre and Casco [4] and Galetto [13].

Morphometric studies of larval stomach/fundus were carried out both in *R. arenarum* (immunhistochemical, IHC) and *X. laevis* (ultrastructural) to analyze the molecules involved in AJs dynamics (See Izaguirre [19]; Galetto [13] for details).

RESULTS AND DISCUSSION

It is very well known that THs exert profound effects on tissues, modulating cell-type dependent proliferation and differentiation [2,4,5]. These are regulated by genomic and non-genomic mechanisms [20,21].

Analogous to the positive T3-responsive control gene (RTβ), E-cadherin, β- and α-catenin genes are upregulated at 24 hrs. A similar performance is exhibited by the Rac1small GTPase. On the contrary, occludin and Rap1 expression become more significant at day 5 of T3-treatment. Similar to IFABP behavior, a negative T3-responsive gene, the Rho small GTPase decreased at day 5 of T3-treatment. Thep120-catenin, Arp2 actin-nucleation protein, Cdc42 and ZO-1 mRNA levels remain practically unchanged both at 24 hours and day 5 of T3-treatment, as well as during spontaneous metamorphosis.

Moreover, putative TERs were found in *X. laevis* E-cadherin, β-catenin, α-catenin and Rac1 genes, but not in the p120-ctn gene [13]. This finding coincides with gene expression responses as early as 24 hrs after T3-treatment. The morphometric analysis in *R. arenarum* coincides with cadhesome-proteins increase despite gastrointestinal intense remodeling (Figure 1). While one E-cadherin-TER was found in a 5´UTR region, one TER was found for β-catenin in intron-1, three TERs for α-catenin arranged in a 5´UTR region and in intron-1, and one site for Rac1 in intron-1.

The duo-expression analysis of guanine nucleotide exchange factors (GEFs) and GTPase-activating protein (GAP) for each small GTPase —Rac1-TIAM/GAP12 pair; Rap1-C3G/SPA1 pair; Cdc42-FRG/Rich pair; RhoA-GEF18/p190-GAP— was less conclusive, but their genes do not respond directly to T3. Only Rac1-GAP12 showed a significant physiological increase at 5 days of T3-induction correlated with decrease of Rac1 and increase of Rap1. These results mainly suggest non-genomic control mechanisms on their GEFs/GAPs and/or others involved.

Morphometric analysis provided very relevant data. While the numbers of tight junctions (TJs) are not modified during T3-treatment, supporting their role in the maintenance function of the epithelial barrier from larval stages to juvenile stages, adherens junctions (AJs) and desmosomes (Dms) led the major changes in epithelial remodeling. At 24 hrs of T3-treatment while AJs number remain constant, Dms significantly decreased. However, the cell-cell distance of AJs and Dms

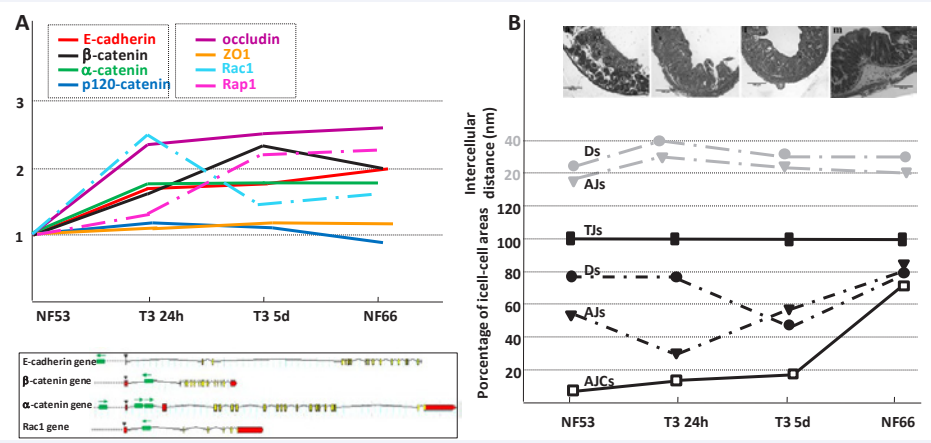
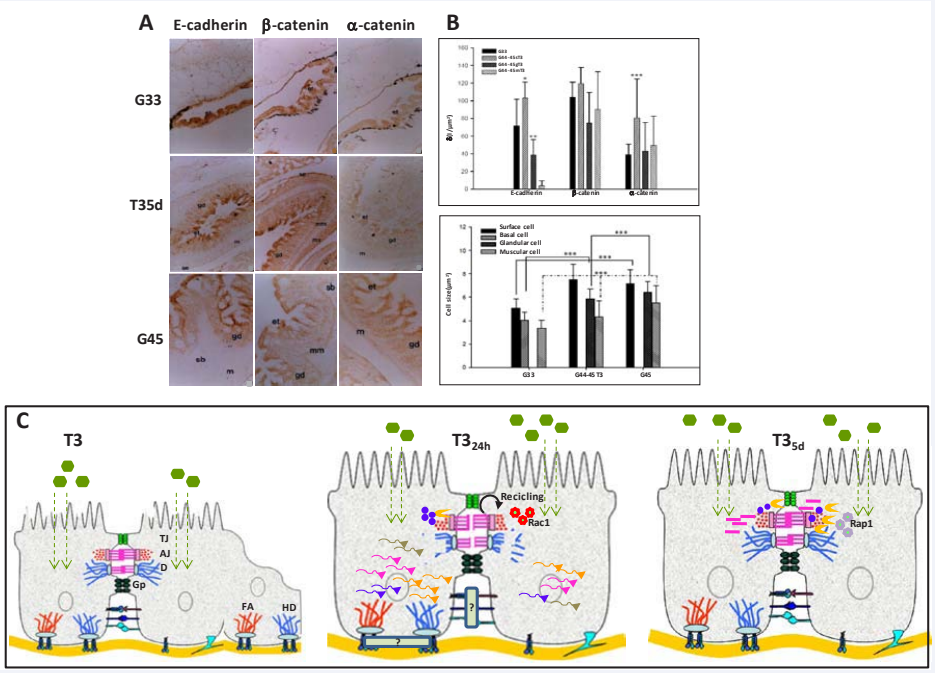


Figure 1 (a) Upper view, representation of X. laevis mRNA expression involved in adherens and desmosome junctions. Values upper to 1.5-fold change respect to those NF53 are considered both physiologically and statistically significant data. Box shows the putative TREs (green box) found in the T3-responsive genes at 24 hrs. (b) Figure shows the stomach histology, the distribution of cell-cell junctions and intercellular distances through spontaneous and T3-induced metamorphosis. NF53: Nieuwkoop and Faber 53larval stage; NF66: Nieuwkoop and Faber 66larval stage; T324h: T3-treated larvae at 24 hs; T35d: T3-treated larvae at 5 days; TJs: tight junctions; AJs: adherens junctions; Ds: desmosomes; AJCs: apical junctional complexes; TREs: thyroid hormone response elements.



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significantly increased, suggesting the increase of epithelial adhesive plasticity, promoting cell proliferation and migration during gastrointestinal remodeling. At 5 days of T3-induction in agreement with a differentiated epithelium, the cell-cell distances of AJs and Dms return to those of mature epithelia, now of juvenile anurans. In contrast, a significant decrease of AJs and a significant increase of Dms were produced correlated with an impressive increase of apical complex junctions (ACJs), features of epithelial barrier strengthening (Figure 1). In addition, the morphometric IHC analysis has demonstrated that T3 exerts a positive regulatory effect on E-cadherin and β- and α-catenin expression and *de novo* synthesis in stomach epithelium during metamorphosis (Figure 2).

CONCLUSION

T3 mediates genomic response on E-cadherin, β-, α-catenin and Rac1 gastrointestinal genes rapidly responding to adhesive plasticity and promoting lamellipodia formation, necessary during epithelial remodeling. In contrast, the master regulator of junctional E-cadherin stability, p120-catenin does not respond to T3, whereas Rap1 indirectly reacts to T3 during the re-establishment of mature epithelium.

These behaviours open the possibility for alternative treatments to control proliferative disorders as colon cancer.

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