The thyroid hormone antagonizes STAT3-dependent transcription in hepatocarcinoma cells

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ABSTRACT

The known functions of the thyroid hormones in the liver have expanded from their well-known roles in regulating metabolism to also participate in liver regeneration, senescence and hepatocarcinogenesis. The transcription factors STAT3 and NF-kB play a key role in liver homeostasis, in the response to infection and inflammation and in hepatocarcinoma development. Interleukin 6 (IL-6) and Tumour Necrosis α (TNFα) are the best-known cytokines responsible for hepatic stimulation of these signalling pathways. We have analysed the effect of thyroid hormones in the response of hepatocarcinoma cells to these factors. We found that triiodothyronine (T3) suppresses IL-6 signalling in cultured Hep3B cells, inhibiting the activation of the main cytokine downstream targets: STAT3 and ERK. In contrast, no inhibitory effects of T3 in the NF-kB response to TNFα were observed. In agreement with these results, T3 strongly antagonized IL-6 stimulated activity of a reporter plasmid bearing STAT-binding elements, while the hormone did not reduce activation by TNFα of a reporter plasmid containing NF-kB binding sites. Transcript levels of the IL-6 receptor or Gp130, essential for IL-6 signal transduction were not altered by T3, but the hormone significantly reduced stimulation by IL-6 of STAT target genes encoding acute-phase proteins, which are key components of the hepatic response to the cytokine. In chromatin immunoprecipitation assays, T3 significantly reduced STAT3 recruitment to its target promoters in response to IL-6. Moreover, IL-6 dependent increase of acetylated histone H4, a marker of transcriptional activation, was also suppressed by T3. Our results show that the thyroid hormones can counteract the cellular responses to IL-6 reducing its transcriptional actions. Through this mechanism, the thyroid hormones may modulate immune homeostasis and carcinogenesis in the liver, suggesting that they could be important targets for developing new therapeutic strategies for the treatment of liver diseases.

T3 antagonizes IL-6 but not TNFα signaling in hepatocarcinoma cells

Figure 1. A. Proteins were extracted from Hep3B cells pretreated with 5 nM T3 in 10% thyroid hormone-depleted serum for 36h and then treated with 10ng/ml IL-6 for times ranging from 0 to 120 min. The levels of phosphorylated and total STAT3 and ERK were assessed by Western blot. **B.** Western blot analysis of the indicated proteins of the NF-κB pathway in cells pretreated with T3 and then incubated with 10ng/ml TNFα for varying times.

T3 inhibits STAT3 transcriptional activity but does not affect NF-κβ activity

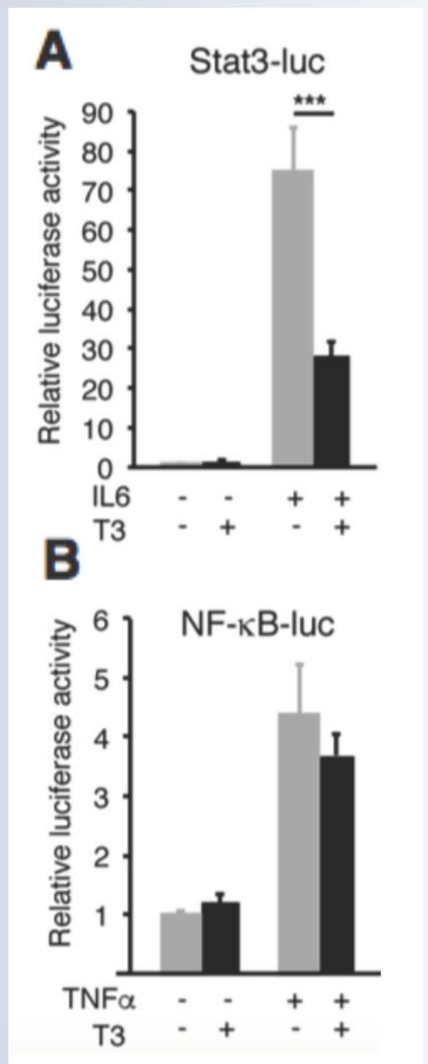

Figure 2. A. Hep3B cells were transiently transfected with a reporter plasmid containing STAT binding sites and incubated in the presence and absence of T3 for 36h and with IL-6 for the last 5h. **B.** Luciferase activity in cells transfected with a reporter plasmid containing NF-κβ binding sites and treated with T3 for 36h and/or TNFα for the last 5h.

T3 represses transcriptional activation of STAT3-dependent genes by IL-6

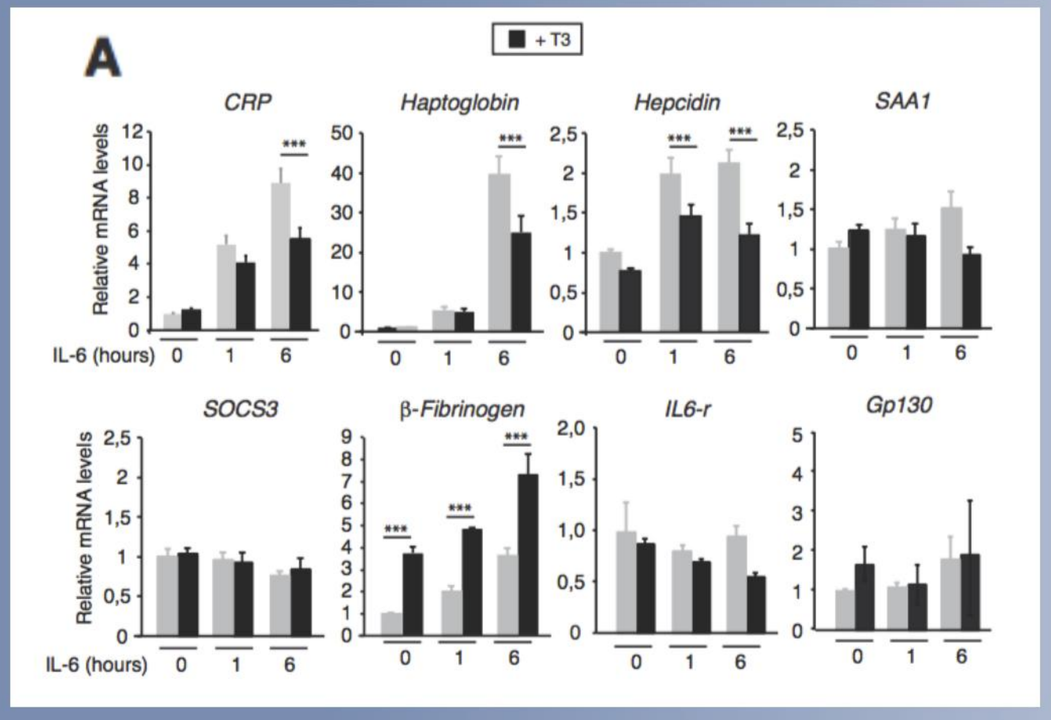

Figure 3. A. Levels of the indicated transcripts (means ±s.d) determined in Hep3B cells treated with 5nM T3 for 36h and with IL-6 for 0, 1 and 6 h. Significance of post-hoc ANOVA test between cells treated with and without T3 is indicated.

T3 reduces STAT3 recruitment and histone 4 acetylation at IL-6 responsive promoters

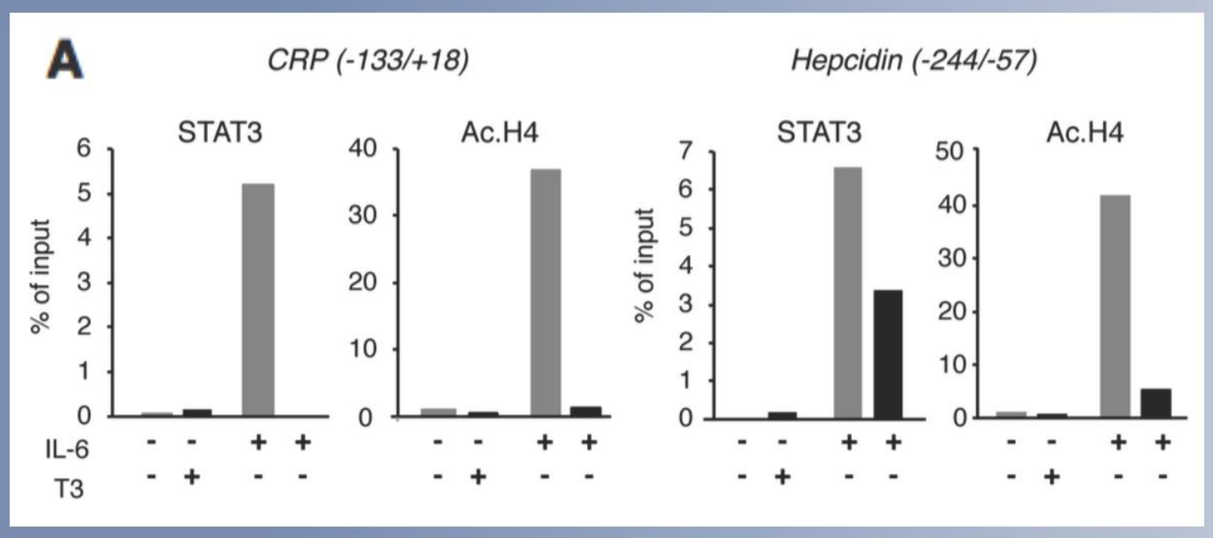

Figure 4 A. Chromatin immunoprecipitation (ChIP) assays with the indicated regions of the *CRP* and *Hepcidin* genes and antibodies against STAT3 or acetylated H4 (Ac.H4). Data are expressed of % of the input after subtracting the values obtained with a control IgG.

CONCLUSIONS

- **The thyroid hormone T3, suppresses IL-6 signaling, inhibiting the activation of the main downstream targets of the cytokine: STAT3 and ERK.**
- **T3 has no effect on the NF-κB response to TNFα.**
- **The hormone antagonizes the induction of acute-phase proteins (APP) genes by the cytokine (IL-6).**
- **Transcripts of the IL-6 receptor or Glycoprotein 130 (Gp130), the common subunit of the type I cytokine receptors, essential for IL-6 signal transduction were not altered by T3.**
- **T3 reduces STAT3 binding to IL-6 responsive promoters.**

The thyroid hormones can antagonize IL-6 dependent transcription, potentially modulating the effects of the cytokine on immune responses and carcinogenesis .

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