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Thyroid hormone-dependent epigenetic suppression of herpes simplex virus-1 gene expression and viral replication in differentiated neuroendocrine cells

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ABSTRACT

A global HSV-1 gene repression occurs during latency in sensory neurons where most viral gene transcriptions are suppressed. The molecular mechanisms of gene silencing and how stress factors trigger the reactivation are not well understood. Thyroid hormones are known to be altered due to stress, and with its nuclear receptor impart transcriptional repression or activation depending upon the hormone level. Therefore we hypothesized that triiodothyronine (T_3) treatment of infected differentiated neuron like cells would reduce the ability of HSV-1 to produce viral progeny compared to untreated infected cells. Previously we identified putative thyroid hormone receptor elements (TREs) within the promoter regions of HSV-1 thymidine kinase (TK) and other key genes. Searching for a human cell line that can model neuronal HSV-1 infection, we performed HSV-1 infection experiments on differentiated human neuroendocrine cells, LNCaP. Upon androgen deprivation these cells undergo complete differentiation and exhibit neuronal-like morphology and physiology. These cells were readily infected by our HSV-1 recombinant virus, expressing GFP and maintaining many processes iconic of dendritic morphology. Our results demonstrated that differentiated LNCaP cells produced suppressive effects on HSV-1 gene expression and replication compared to its undifferentiated counterpart and T_3 treatment has further decreased the viral plaque counts compared to untreated cells. Upon washout of the T_3 viral plaque counts were restored, indicating an increase of viral replication. The qRT-PCR experiments using primers for TK showed reduced expression under T_3 treatment. ChIP assays using a panel of antibodies for H3 lysine 9 epigenetic marks showed increased repressive marks on the promoter regions of TK. In conclusion we have demonstrated a T_3 mediated quiescent infection in differentiated LNCaP cells that has potential to mimic latent infection. In this HSV-1 infection model thyroid hormone treatment caused decreased viral replication, repressed TK expression and increased repressive histone tail marks on the TK promoter.

1. Introduction

HSV-1 has been found in 80% of US adults through serological testing [1]. A number of studies have identified HSV-1 DNA in over 90% of adults that are 60 years of age and above [2–4]. Interestingly many carry the virus yet the unsightly and painful oral lesions are not equally as prevalent. This phenomenon can be attributed to certain characteristics of the virus and complex host immune system virus interactions. One particular characteristic that herpes viruses including the ones that cause chicken pox, shingles, mono and several cancers, have developed methods persisting within its host undetected yet still able to spread to new hosts from time to time. For example, HSV establishes latency in sensory neurons and is characterized by the lack of viral replication and gene expression. Throughout the life of an infected host, events such as stress, local trauma, and surgery may trigger viral replication [5]. Hormone fluctuations have been suggested to play a role in HSV reactivation [5] and is known to oscillate due to stress and regulates many cellular functions in neurons; we hypothesized that thyroid hormone may play a role to regulate HSV-1 replication and gene expression in differentiated neuronal environment.

Thyroxine (T_4) and its physiologically active metabolite 3,5,3-triiodothyronine (T_3) are collectively called thyroid hormones (TH). They are involved in a myriad of biological functions ranging from brain development and function to metabolism, immune system activation and cardiac rhythm [6]. The mechanisms that drive these TH mediated biological events are extensively described, including the transcriptional regulation via nuclear TH receptor (TR) as well as the non-genomic TR effects modulating signaling pathways [7]. The most well investigated transcriptional regulation by TR involves genes that are down regulated in the absence of TH and activated when TH is bound to TR, which binds to the promoter region of the regulated gene. In this type of regulation,

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TR binds promoter regions, as a homodimer or as a heterodimer with retinoid-X-receptor (RXR), at specific DNA sequences known as positive TR elements (pTREs) [8,9]. The most common form of pTREs is composed of a pair of six-nucleotide sequence AGGT(G/C)A with a four nucleotide long spacer, which is known as a direct repeat four (DR4) [10]. In the absence of TH a TR dimer bound to a DR4 is in the appropriate conformation to interact with co-repressor complex, SMRT or NCoR and further recruit histone modifying enzymes that can remove active transcriptional marks and add repressive modifications to histone tails associated to the regulated gene and its promoter for transcriptional repression. Following TH binding to TR, TR undergoes a conformational change that ejects co-repressor complexes and recruits co-activator complexes that include histone modifying enzymes that reverse the effects of the repressor complexes [11].

Like many biomolecules THs exhibit a feedback inhibition mechanism at several key steps within the TH synthetic signaling pathway. These mechanisms have also been shown to be mostly TH dependent negative regulation [11]. The exact mechanisms involved in the negative regulation for the handful of genes during the feedback events have been studied for many years without much conclusion or consensus [12]. For example, thyroid stimulating hormone gene α (TSHα) exhibits TH dependent transcription repression but the correlated negative TRE (nTRE) was poorly defined and showed different regulation pattern in different cellular environments [13–15]. It has been shown that T_3 can either positively or negatively regulate viral gene promoters such as Human Immunodeficiency Virus (HIV) LTR [16–18] and Herpes Simplex Virus 1 (HSV-1) Thymidine kinase [13,19].

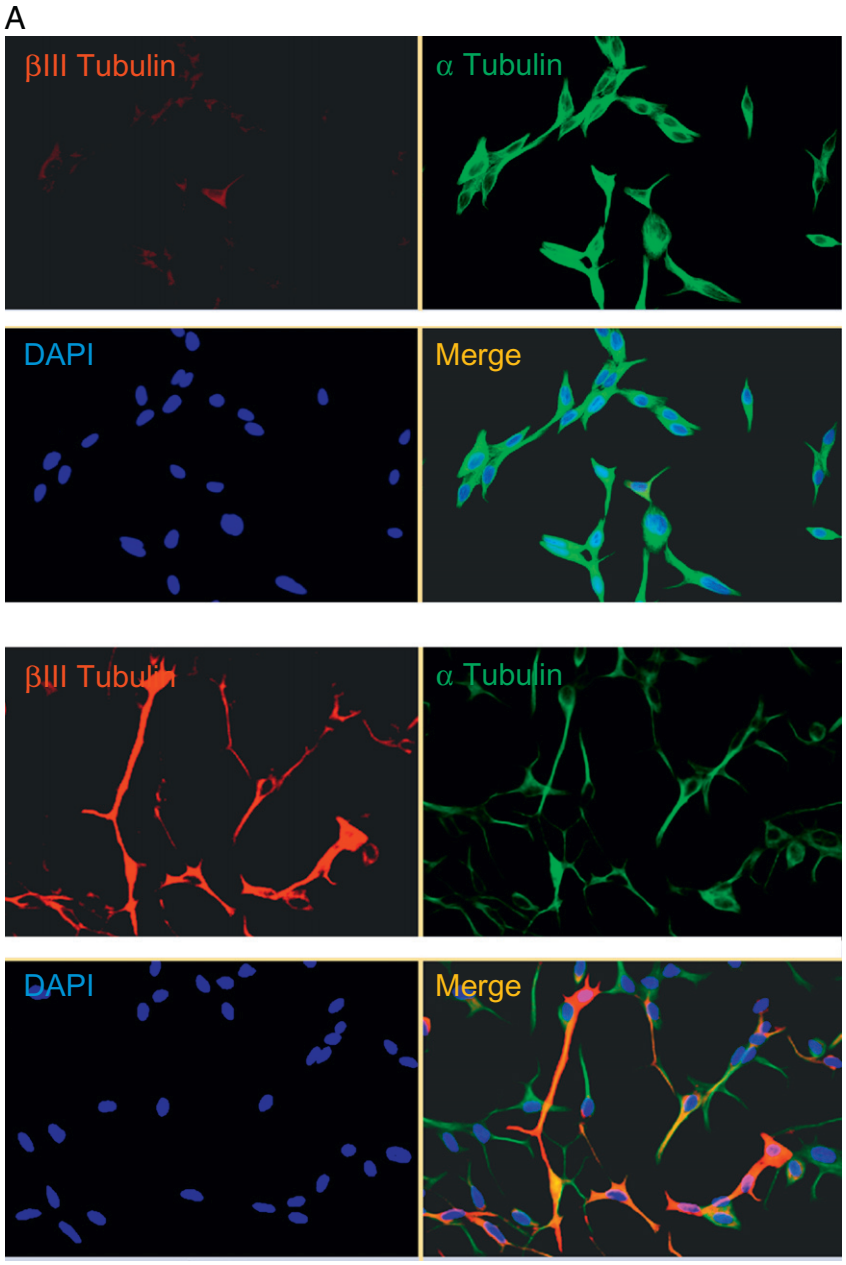

Fig. 1. Differentiation of LNCaP cells and viral infection. A. Undifferentiated LNCaP cells were compared to differentiated ones by immuno-fluorescent microscopy using neuronal maker β-tubulin (red) and control α-tubulin (green). It is noted that differentiated LNCaP cells undergo morphological changes with expression of β-tubulin. B. Differentiated cells were infected by recombinant HSV-1 expression GFP. It can be seen that infected cells expressed fluorescence and can survive a week at moi of 1.