論文の内容の要旨

Dissertation Abstract

論文題目 **Dissertation Title:**

STUDY ON HOST FACTORS REGULATED BY TAX PROTEIN OF HUMAN AND BOVINE RETROVIRUSES

(ヒトおよびウシレトロウイルス転写活性化因子 **Tax** タンパク質によって制御される宿主因子の解析**)**

氏名　マリルース　アナメルバ　アラインガ　ラミーレス

1. Introduction

Human T-cell leukaemia virus types 1 (HTLV-1) and bovine leukaemia virus (BLV) are closely related retroviruses which can develop leukaemia. Both viruses encode regulatory proteins involved in viral infection and regulation of viral expression, such as Tax, which was originally identified as transcriptional transactivator of viral expression. In HTLV-1 and BLV, Tax are thought to be involved in cellular processes that include cell growth and proliferation, transformation and immortalization, cell cycle progression, apoptosis, cellular DNA repair and cell survival. HTLV-1 Tax is known to modulate the expression of many cellular genes that are related to regulation of cell growth, but little is known about BLV Tax.

Aside from the difference in host range, a notable difference between the two viruses is that infection by BLV is associated with malignancy of B cells, whereas HTLV affects T cells. In this study, to gain a better understanding of the contribution of Tax to HTLV-1- and BLV-induced pathogeneses, genes that play a role in the cascade of signal events regulated by HTLV-1 or BLV Tax were identified in HeLa cells by microarray-based gene expression analysis; and, the regulation of cell cycle and apoptosis by HTLV-1 Tax was visualized by time-lapse imaging.

2. Results

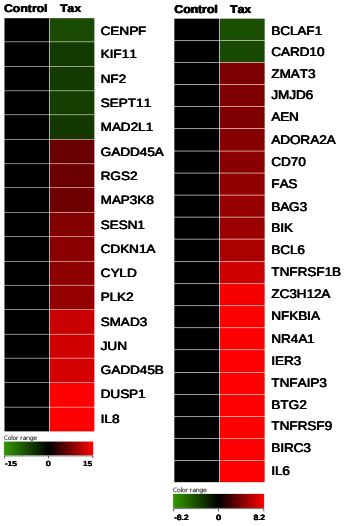
2-1 Host factors regulated by HTLV-1 Tax

Studies about the retroviral protein HTLV-1 Tax have been focused on the function of Tax in regulation of cell cycle and cell proliferation. Expression of Tax has been previously shown to regulate cell cycle progression and apoptosis both positively and negatively, while the molecular mechanisms underlying the regulation of these processes by HTLV-1 Tax remains obscure.

2-1-1 Expression profiling of cellular genes following expression of HTLV-1 Tax

To first know the biological characteristics of HTLV Tax, expression vector encoding Tax was constructed and transfected into HeLa cells. I found that Tax can induce cell cycle arrest at the G_1 phase in HeLa cells by flow cytometry analysis and by analysis of the phosphorylation status of Retinoblastoma. Moreover, analysis of Annexin V staining and caspase-3 activity clearly demonstrated that Tax promotes apoptosis.

To analyze the transcriptional effects of HTLV-1 Tax on global gene expression, I performed microarray analysis in HeLa cells and identified 342 genes (269 were up-regulated and 73 were down-regulated) showing differential regulation by >2 fold. The up-regulated genes were clustered within functional groups involved in transcription/translation/RNA processing, signal transduction, immune response, apoptosis, cell cycle and cell growth/proliferation. In particular, a number of molecules involved in immune response were significantly down-regulated by Tax.

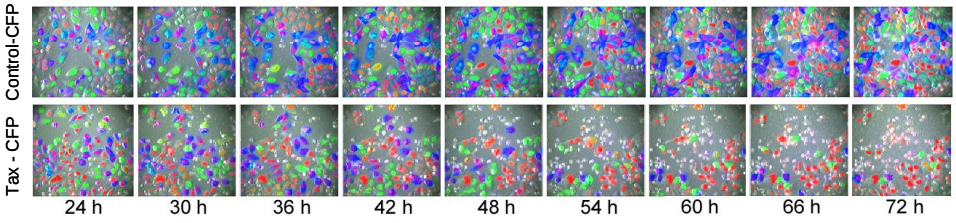


1A **1B**

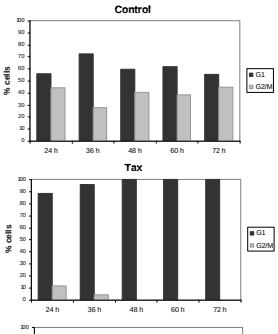
I next focused on the mechanism by which Tax induces cell cycle arrest and apoptosis. I identified 17 Tax-dependent genes related to cell cycle regulation resulting in >2.0 fold up- or down regulation (Fig. 1A) and they were involved in response to stress and DNA damage, cell proliferation, mitotic cell cycle and inflammatory and immune response, such as SMAD3, JUN, GADD45β, DUSP1 and IL8. Additionally, 23 pro- and anti-apoptotic genes were deregulated by Tax, including TNFAIP3, TNFRS9, BIRC3 and IL6 factors (Fig 1B). Moreover, microarray results were confirmed by performing qRT-PCR on five up-regulated genes.

2-1-2 Visualizing dynamics of cell cycle and apoptosis regulations by Tax protein.

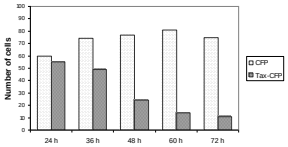
2A



2B



2C



To clarify whether Tax causes apoptosis independently of its ability to induce G_1 arrest, the spatiotemporal patterns of cell cycle regulation in response to Tax expression were visualized in HeLa/fluorescent ubiquitination-based cell cycle indicator (Fucci) cells which allows for dual-color imaging and can be used to distinguish between live cells in the G_1 and the $S/G_2/M$ phases. I monitored that Tax-induced cell death after cell cycle arrest at G_1 phase (Fig. 2A). A drastic increase was observed in the proportion of Tax-

expressing cells at G1 phase, as compared with control cells (Fig. 2B). Interestingly, as shown in Figure 2C, overexpression of Tax seemed to reduce the number of HeLa/Fucci2 cells in culture.

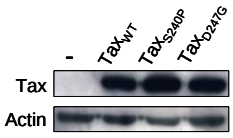
2-2 Host factors regulated by BLV Tax

Our group have been previously identified a super-activator form of BLV-Tax (Tax_{D247G}) and an attenuated BLV-Tax (Tax_{S240P}) that exhibit impaired transactivation activity. However, the effects of these mutations on functions other than transcriptional activation are unknown. Therefore, I performed a comparative analysis to identify the functional differences between wild-type and mutants BLV-Tax.

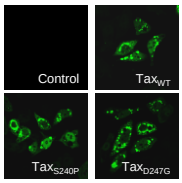
2-2-1 Expression of BLV-Tax and its derivatives in HeLa cells

The expression vectors encoding Tax_{WT}, and mutants Tax_{S240P} and Tax_{D247G} were constructed and transfected into HeLa cells. The protein expression and localization did not differ by any BLV Tax (Fig. 3A-B). The transactivation capacity of Tax showed that Tax_{D247G} induced highest viral LTR-directed transcriptional activation and in contrast Tax_{S240P} displayed markedly reduced activity (Fig. 3C).

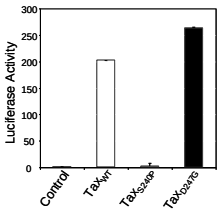
3A



3B



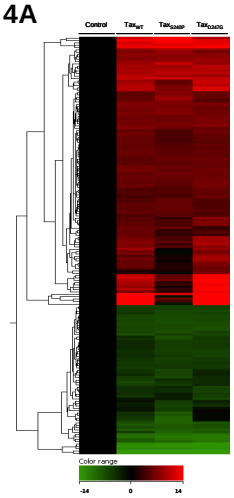
3C



2-2-2 Large-scale expression profiling of cellular genes following expression of wild-type BLV-Tax and its derivatives

To analyze the transcriptional effects of BLV Tax on global gene expression, we performed microarray analysis (Fig. 4A) and identified a total of 122, 118 and 139 genes showing differential regulation by >2 fold upon Tax_{WT}, Tax_{S240P} and Tax_{D247G} expression respectively ($p<0.05$) (Fig. 4B). The genes up-regulated were involved in transcription, signal transduction, cell proliferation, apoptosis, cell adhesion, etc. Interestingly, Tax_{D247G} deregulated more genes belonging to the transcription group; by contrast to Tax_{S240P} which displayed the lowest impact on cellular gene expression. In particular, a number of molecules involved in immune response were significantly down-regulated by all Tax (Fig. 4C).

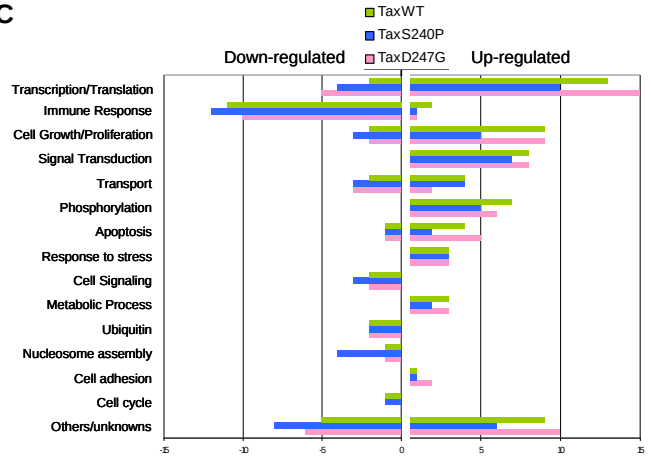
4A



4B

Genes	Tax_{WT}	Tax_{S240P}	Tax_{D247G}
Up-regulated	90	64	95
Down-regulated	32	54	44
Total	122	118	139

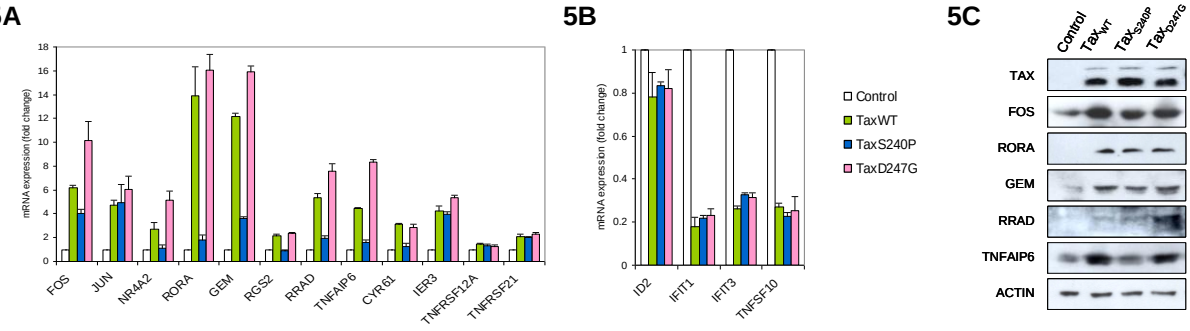
4C



2-2-3 Validation of expression of host cellular genes induced by BLV-Tax

First, qRT-PCR was used to corroborate the fold change obtained from microarray analysis of the up-regulated genes in the categories of transcription/translation/RNA processing, signal transduction, immune response and regulation of cell growth/cell proliferation; confirming that Tax_{D247G} was inducing a strong response while Tax_{S240P} was inducing lower levels of expression than others Tax (Fig. 5A). I also validated the results of the down-regulated genes which correlated perfectly with the microarray analysis (Fig. 5B). Finally, we utilized Western blotting to determine the levels of proteins (Fig. 5C). In general, results were in good agreement with, and support, the findings of our microarray analysis of HeLa cells.

5A **5B** **5C**



3. Discussion

In the case of HTLV-1 Tax study, I have visualized that Tax arrested cells at the G_1 phase of the cell cycle, thereby inducing apoptosis in HeLa cells and demonstrated that Tax mediates cellular factors with respect to cell cycling and pro- and anti-apoptosis as well as inflammatory and cancer diseases. To our knowledge, this study is the first to highlight the morphological dynamics of Tax-induced cell death after cell cycle arrest at the G_1 phase.

Using interesting variants forms of BLV Tax, with elevated (Tax_{D247G}) or reduced (Tax_{S240P}) transactivation activity on the replication and propagation of BLV; I identified cellular genes, which were importantly involved mainly in transcription, signal transduction, cell growth, stress response and immune response. Interestingly, there were differences in the cellular gene expression between the three Tax variants, with emphasis in BLV-Tax_{D247G} which had the ability to induce large numbers of host genes to high levels, contrary to BLV-Tax_{S240P} which induced less numbers of genes and only to relatively low levels. These results suggest the correlation of gene expression deregulation with the transactivation properties of these BLV-Tax alleles. My study also shown the discovery that BLV-Tax and its derivatives profoundly down-regulated the expression of genes involved in the innate immune response.

This overview can be extended into Tax-mediated signalling and further study of the interactions of Tax and cellular factors will provide insights into the mechanisms by which HTLV-1 and BLV Tax can regulate specific cell behaviours.

4. Conclusions

HLTV-1 Tax induced cell cycle arrest at G1 phase and undergone to apoptosis and these events where in correlation with the expression of genes involved in both functions.

BLV Tax variants deregulated the expression of cellular genes, which were importantly involved mainly in transcription, signal transduction, immune response and cell proliferation.