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Effects of specific nutrients on tax-dependent activation of NF-κB and MMP-9 in human T-cell lymphotropic virus -1 positive malignant T-lymphocytes

Steve Harakeh[1*], Mona Diab-Assaf[2], Rania Azar[2], Esam Azhar[1,3], Ghazi A. Damanhouri[1], Adeel Chaudary[1,3], Haitham Yacoub[4], Mourad Assidi[5], Muhammad M. Abu-Elmagd[5], Mohammed H. Alqahtani[5], Adel M. Abuzenadah[5], Taha Kumosani[1], Aleksandra Niedzwiecki[6], Mathias Rath[6], Elie Barbour[7]

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Background

Adult T-cell Leukemia (ATL) is a disease with no known cure so farand it is a resistant to chemotherapy. The virus can be transmitted by exchange of bodily fluids through the placenta and from mother to child. Only 5% of those who are infected develop the disease after a long latency period ranging from 30-50 years [1,2]. The disease manifests itself as an aggressive proliferation of $CD4^+$ cells with the human T-cell Lymphotropic virus type 1 (HTLV-1) [3]. The leukemogenesis of the virus is mainly attributed to the viral oncoprotein, Tax, that activates the Nuclear Factor kappa B (NF-κB) which in turn stimulates the activity and expression of the matrix metalloproteinase-9 (MMP-9)which is important in angiogenesis[2]. Our previous work has shown that using non-cytotoxic concentrations of a Specific Nutrient Synergy (SNS)mixture resulted in the induction of apoptosis in both HTLV-1 positive and negative malignant T-lymphocytes [1]. The objective of this study is to investigate the efficacy of SNS on Tax expression, NF-κB levels as well as on MMP-9 activity and expression both at the transcriptional and translational levels intwo HTLV-1 positive cell lines, HuT-102 and C91-PL.

Materials and methods

Cell growth, experimental design, source of SNS, preparation and storage of stock solution, were previously described by our group [1].The effects of non-cytotoxic concentrations of SNS ranging from 0-350 μg/ml were evaluated for their efficacy on proliferation, Tax expression, NF-κBmobility and the activity and expression of MMP-9 at 48h and 96hof incubation.Cytotoxicity of EGCG was assayed using CytoTox 96 Non-radioactive and proliferation was measured using Cell Titer96™ Nonradioactive Cell Proliferation kit(MTT- based assay). Elisa and EMSA were used to assess the effect of SNS on NF-κBmobility. Zymography was used to determine the effects of SNS on the activity and secretion of MMP-9. The expression of MMP-9 was done using RT-PCR at the translational level and Immunoblottingat the transcriptional level.

Results

A significantinhibition of proliferation was seen in both cell lines starting at a concentration of 200μg/ml and in a dose dependent manner. SNS induced a dose dependent decrease in Tax expression (Fig.1),which was paralleled by a down-regulation of the nuclearization of NF-κB (Fig.2). This culminated in the inhibition of the activity of MMP-9 and their expression both at the transcriptional and translational levels (Fig.3).

* Correspondence: sharakeh@gmail.com
[1]Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
Full list of author information is available at the end of the article

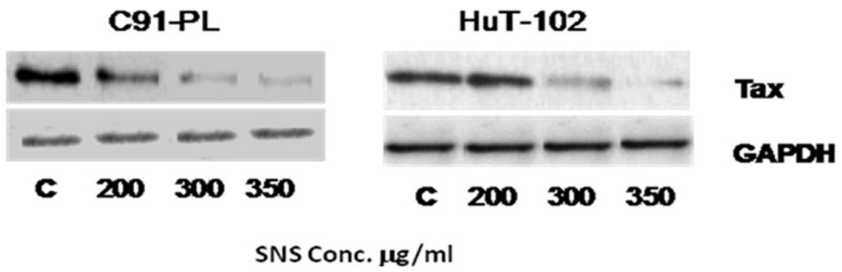

Figure 1 Effect of SNS on Tax expression in C91-PL and HuT-102 cell lines. Equal loading was ensured using GAPDH. The immunoblots represent results obtained in one of three independent experiments.

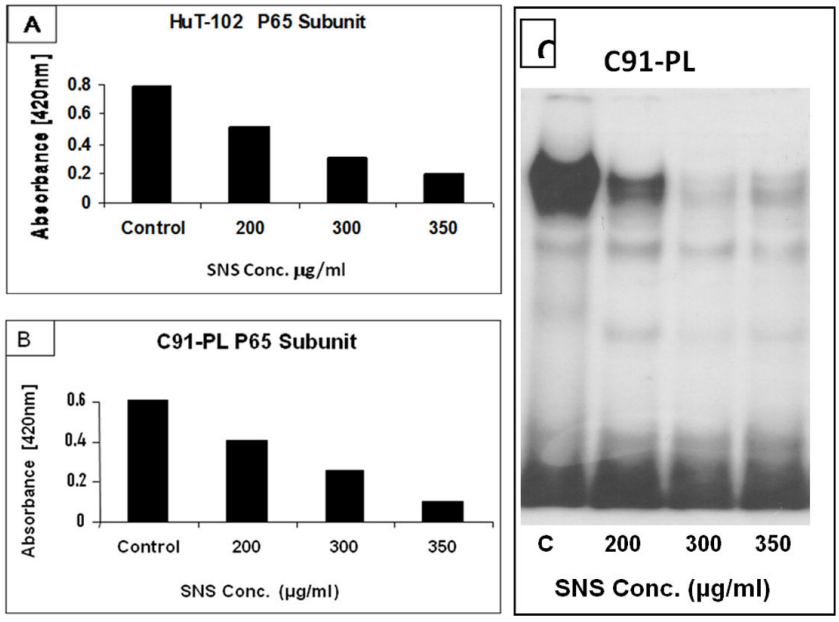

Figure 2 Effect of SNS on NF-κB nuclear translocation in HuT-102 and C91-PL HTLV-1 positive cell lines. (A,B) Nuclear levels of P65 subunit of NF-κB was evaluated by ELISA at 48 and 96 h of incubation. Each value is the mean ± SD deduced from three separate experiments done in triplicate. (C) EMSA Gel representing one of three independent experiments with nuclear extracts of C91-PL.

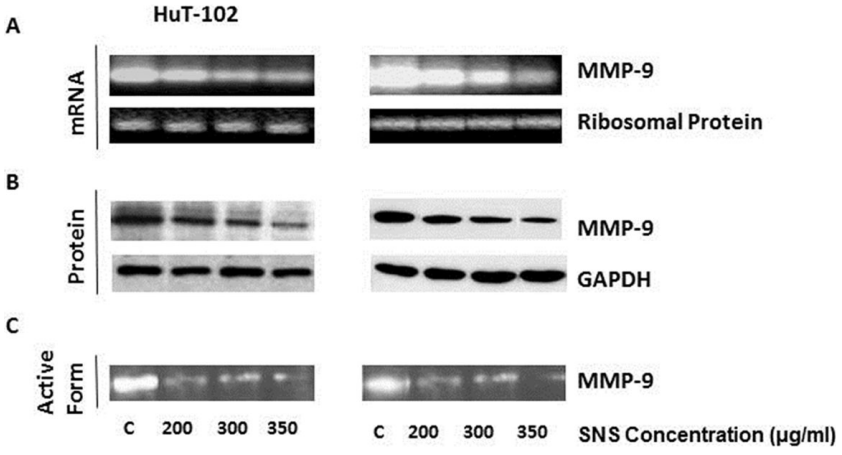

Figure 3 Effect of SNS on MMP-9 mRNA (a), protein (b) and activity (c) in two ATL-HTLV-1 positive cell lines. Equal loading was ensured using ribosomal protein for mRNA expression (a) and GAPDH for protein expression (b).The results represent one out of three independent experiments.

Conclusions

The role of nutrients in the treatment of disease has been overlooked for a long time. Recently, it has been recognized that nutrients play a crucial role in the outcome of the treatment. The results of this study indicate that a specific nutrient synergy targeted multiple levels pertinent to the progression of ATL. Its activity was mediated through the NF-κB pathway, and hence has the potential to be integrated in the treatment of this disease as a natural, yet potent anticancer agent.

Authors' details

[1]Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. [2]Molecular Tumorigenesis and Anticancer Pharmacology, Lebanese University, Hadath, Lebanon. [3]Department of Medical Laboratory technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. [4]Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. [5]Center of Excellence in Genomic Medicine Research, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. [6]Dr. Rath Research Institute, Santa Clara, CA, USA. [7]Department of Animal and Veterinary Sciences, American University of Beirut, Lebanon.

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