CHARACTERIZATION OF THE ANTI-VIRAL ACTIVITY OF SCHLAFEN13

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THESIS

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ABSTRACT

Schlafen13 (Slfn13) is an enzyme that belongs to the Schlafen family whose expression and function is not very well characterized. The N-terminal has a pseudo dimer structure that contains its catalytic site. There is no characterization functionally or structurally of the C-terminal of Slfn13 other than the prediction of a region with helicase activity. **The objective of my thesis was to increase our understanding of the Slfn family of proteins**. Currently Slfn13 is reported to play a role in the differentiation of monocytes and to function as an endoribonuclease that cleaves tRNA and rRNA molecules in a site dependent sequence independent manner. Further analysis of the anti-viral activity is hampered by the poor expression of the full-length protein. We have been unable to detect Slfn13 by western blot using different antibodies in different cell lines which were successfully transfected either stably or transiently. Our data indicates that regulation of Slfn13 expression is at a post-transcriptional level. We demonstrated the robust expression of endogenous and exogenous mRNA encoding Slfn13 in different cell lines by means of RT-PCR. This suggests a potential instability and/or toxicity of Slfn13. We investigated whether regulation is at the post translational level by using compounds that inhibit proteasomes and lysosomes. Despite obstructing these major pathways of protein degradation, we were unable to rescue Slfn13 expression. It is reported that type I-interferons play a role in the induction of Slfn proteins. Intrinsically, we theorized that perhaps an interferon-induced chaperon was required for Slfn13 expression but interferon α failed to stabilize this protein. In contrast to Slfn13, the family member Slfn11 is widely expressed at very high levels in multiple cell lines. We took advantage of the evolutionary conservation between these two proteins and swapped the last third residues of Slfn13 with those of Slfn11. This chimera was readily expressed indicating that the regulation was post-translational. Furthermore, this indicates that the last third of Slfn13 is responsible for the instability of this protein. Since the Slfn11 portion in the chimera does not have anti-viral activity, we also investigated the activity of Slfn13/11 Chimera against HIV-1 and flaviviruses. Our findings indicate that the Slfn13/11 chimera lacks anti-viral activity. However, we cannot ascertain the presence or absence of Slfn13 through normal methods. We believe that localization plays a role in the expression of this protein as the c-terminal of region of Slfn11 in the Slfn13/11 contains a predicted nuclear localization sequence. This led us to probe which of the last third of the c-terminal is responsible for the instability of this protein and how we could exploit this feature for molecular biology purposes. **In summary, because the Slfn13/11 Chimera is 80 percent identical to Slfn13 and it is known that c-terminal has no activity; we can say that Slfn13 also lacks anti-viral activity.**

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INTRODUCTION

Viruses require exploiting cellular resources for replication and the immune system thwarts these strategies by targeting these resources. In this study I will focus on two group of evolutionarily distant viruses, HIV-1 and *Flaviviruses*, and the activity of members belonging to a family of type I interferon-induced proteins called Schlafen. The relevance of this study resides on the medical significance of these pathogens that I describe in the next pages.

Medical Relevance of HIV-1 and *Flaviviruses*. Presently, there is no vaccine for HIV. Anti-retroviral therapy is very efficient at controlling viral replication[1]. These drugs are categorized by inhibition of integration, reverse transcription, and protease cleavage[1]. However, there are concerns about toxicity and side effects related to required long term use and drug-drug interactions[2,3]. Individuals receiving anti-retroviral therapy are often administered a cocktail of drugs that must be administered for the remainder their lives[2,3]. Studies illustrate that a combination of drug interactions and overall toxicity from long term use lead to a variety of disorders[2,3]. These disorders are generally associated with co-morbidities of HIV infected individuals and are in the realm of metabolic complications but can also be neurological and cardiovascular[2–4]. The adverse effects of nucleoside reverse transcriptase inhibitors (NRTI) are attributed to these anti-viral drugs blocking mitochondrial DNA polymerase leading to mitochondrial toxicity that induce metabolic disorders[5]. Integrase strand transfer inhibitors (ISTI) induce a decrease in insulin sensitivity certain individuals[2,3]. Lipodystrophy and type 2 diabetes is a common indication of the adverse metabolic effect of antiviral drugs[1-3]. In addition, it is reported that protease inhibitors not only contribute to the same metabolic disorders as NRTIs and ISTIs but also increases rate of heart failure mortality[4]. Antiretroviral treatment is also associated with increased risk of neuropathy that is present in the form of decreased motor

function and neurocognitive impairment[6].

Regarding *Flaviviruses*, there are no anti-viral options in clinical use to combat infection of any viruses belonging to the genus[7]. There are currently vaccines for several including yellow fever, Japanese encephalitis, and Dengue virus[7]. While the vaccines for yellow fever and Japanese encephalitis appear to be efficient and safe, administration of the dengue virus vaccine has been limited due to complications in certain groups of individuals[7]. Studies demonstrate that the vaccine protects against dengue virus for an average of five years in individuals with prior exposure to the pathogen[8]. However, vaccinated individuals with no prior exposure are at higher risk of being hospitalized for dengue than those who have not been vaccinated[8]. As for West Nile virus (WNV) and Zika virus (ZKV), no vaccines exist[7]. Treatment for those infected with these viruses is limited to pain management and intravenous fluids[7]. The medical implications relating to HIV and flaviviruses demonstrate the need for further research relating to these pathogens.

HIV Pandemic. Acquired Immunodeficiency syndrome (AIDS) is a global threat[9]. It was classified as a new disease in 1981, after a rise in individuals dying due to unusual circumstances[10]. Human Immunodeficiency Virus (HIV), the virus responsible for the disease was later identified and has become one of the fast spreading infectious diseases[10]. Global spread of HIV has led to its classification as a pandemic with more than 75 million individuals that have been infected since its classification and 37 million infected individuals that live with the virus today[1,10]. Areas with the highest population of infected individuals are seen in third-world and developing nations; the highest of which are located in sub-Saharan Africa[10]. There are two species of HIV, HIV-1 and HIV-2[1,10,11]. Both species belong to the subgroup lentivirus genus of the retrovirus family of positive sense single strand RNA viruses[1,10,11]. The two species of HIV differ only in one of the accessory proteins[11]. It is reported that origins both HIV-1 and HIV-2

can be traced back to simian immunodeficiency virus, with HIV-1 being the more virulent of the two and responsible for the current pandemic[10].

Flaviviruses are Emerging Pathogens. *Flavivirus* infection rates are rising globally. In 2016 there were 4 million ZIKV infections in the Americas alone[12]. DNV infections have increased six-fold between 2010 and 2016[13]. There has been a similar rise in WNV infections between 2014 and 2018. Areas with the highest rate of flavivirus infections much like HIV are developing countries. Rates of infection are particularly the highest in African countries[14]. It is believed that these rates are higher than reported due to the Pathogenesis of flaviviruses where 80 percent of infected individuals are asymptomatic[15,16]. However, there are variances in pathogenesis that lead to different disease prognosis[17,16]. Microcephaly was not reported to be associated with ZIKV until 2013 despite being first discovered in 1947[12,18]. Regarding WNV, 1% of those infected develop neuro-invasive disease[14,15]. The emergence of *Flaviviruses* and the variance in their pathogenesis illustrates the relevance for researching these pathogens

Strategies of Viral Replication. The Baltimore classification system of viruses was characterized by David Baltimore in 1971[19]. This classification also acknowledges the significance of the central dogma of molecular biology in that viruses only carry out two processes[19]. Generating genetic material and mRNA to be used for protein synthesis[19]. As such, it classifies animal viruses based on genome and their mechanisms of replication[19,20]. Class I viruses are classified by having a double strand DNA genome[19,20]. These viruses are much like hosts and give rise to mRNA through transcription of their DNA genome[19,20]. Class II are single stranded DNA genome viruses and produce mRNA in the same manner as Class I viruses[19,20]. Class III viruses consists of double stranded RNA genome viruses[19,20]. They have multiple copies of double stranded RNA and usually produce a single protein from each piece of genetic

information[19,20]. Class IV viruses are single stranded positive sense viruses (ssRNA (+)), their genomes are translated directly to protein[19,20]. These viruses tend to produce a polyprotein from their mRNA that is cleaved into several proteins[19,20]. Some also produce different proteins through sub-genomic mRNA and ribosomal frameshifting[19,20]. It is worthy to note that the mRNA used to produce proteins is identical in base sequence to the viral genome[19,20]. Thus, for these viruses to replicate their genomes they must first produce a negative sense strand of RNA from their genome[19,20]. Class V is comprised of single stranded negative sense RNA genome viruses ssRNA(-)[19,20]. These viruses have a genome that is complementary to the mRNA required to produce viral proteins[19,20]. They require an RNA dependent RNA polymerase to produce a complementary strand of RNA that is positive sense[19,20]. The complementary strand serves as a template for viral genome and produces viral protein[19,20]. Class VI viruses are ssRNA (+) that have a DNA intermediate. They do not use RNA as a template to produce copies[19,20]. The RNA genome is reverse transcribed into DNA which is integrated into the host and then transcribed using host polymerases[19,20].

HIV-1 Structure. The HIV-1 genome consists of two identical copies of 9.7kb single strand positive sense RNAs[21](Fig. 1). There are nine gene products obtained from splicing that code for 9 different proteins, 3 of which are cleaved into several different protein products[11,22]. The genome is divided into structural genes, essential regulatory genes, and accessory genes enclosed by a three prime and five prime long terminal repeats[21,23]. The structural genes of the HIV-1 genome are gag, pol, and env. The envelope gene codes for glycoprotein gp160 which is cleaved to form two glycoproteins gp120 and gp41. The glycoprotein gp41 is a transmembrane protein that spans the lipo-protein bilayer of the envelope made as new virions are budding from infected host cells. A non-covalently bonded heterodimer is formed between gp41 and trimers of gp120.

The gag gene codes for structural proteins p6, p7, p17, and p24[24,25]. Proceeding inward from the viral envelope is the matrix made of p17 followed by the viral capsid made of p24 and the nucleocapsid made of p17. Reverse transcriptase (RT), integrase (IN), RnaseH, and protease (PR) are all proteins that are coded for by the pol gene[11]. PR cleaves protein products, RT's function is to transcribe viral RNA to double stranded DNA, and IN facilitates the integration of viral DNA into the host genome. These structural genes are hallmarks of all retroviruses. Moving on to the essential regulatory elements, the genes tat and rev code for the tat and rev proteins respectively. Tat is said to be involved in the expression of the viral genome while rev functions in exporting messenger viral RNA from the nucleus after processing. The accessory genes are vif, vpu, vpr, and nef. They code for proteins of the same name respectively. Viral infectivity factor protein coded for by the vif gene is involved in the infectivity of HIV-1 in specific cell lines. It is required for the infectivity in some cell lines but not others. Vpu is exclusive to HIV-1 and is not present in HIV-2. This protein's function is involved with the release of viral particles from infected host cells. Vpr assists with the movement reverse transcribed viral DNA into the nucleus and is also involved in cell cycle arrest. Nef, or negative factor is associated with viral budding, and infectivity.

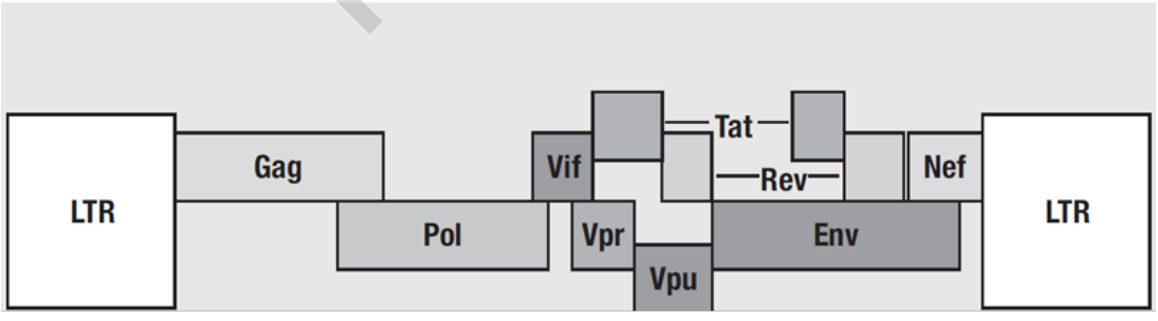

Fig. 1 HIV-1 genome structure.

Emanuele Fanales-Belasio(a), Mariangela Raimondo(b), B. S. and S. B. HIV virology and pathogenetic mechanisms of infection: a brief overview. Clin. Ter. 46, 5–14 (2010)