Abstract

A recent study has shown variable p21 expression levels linked to individuals displaying different levels of HIV-1 control, with elite controllers (ECs) and viraemic controllers (VCs) exhibiting higher p21 expression when compared to both healthy HIV-1 negative individuals and HIV-1-infected progressors. The role of p21 in HIV-1 control in a sub-Saharan African population has not been established.

Polymorphisms in the regulatory regions of p21, as well as in the microRNAs (miRNAs) that affect p21 regulation can contribute to differential p21 expression. In this study we developed real-time PCR assays to genotype the p21 exonic rs1801270 and 3’UTR rs1059234 SNPs, in addition to the p21-associated miRNA (miR-106b) rs999885 SNP. We determined their allelic and genotypic frequencies in Black South African HIV-1 negative individuals (n=72), HIV-1 controllers (HICs) (n=52) further subdivided into ECs (n=11), VCs (n=30) and high viral load long term non-progressors (HVL LTNP) (n=11), and HIV-1 infected progressors (n=74). We sequenced a region of the p21 5’UTR and 3’UTR in a subset of these individuals (HICs: n=52, progressors: n=44) to identify variants that may be modulating p21 expression.

We compared levels of p21 mRNA, a marker for p21 expression, in a smaller group of individuals (n=50) with similar clinical phenotypes to determine if p21 upregulation was associated with natural control of HIV-1. Lastly, we developed a real-time PCR assay to genotype a p21 5’UTR SNP, rs733590, that alone, and together with HLA-B*2705, was recently shown to directly impact on p21 expression in Caucasians. This SNP was genotyped and analysed in the individuals with p21 mRNA expression data.

The p21 rs1801270 and rs1059234 SNPs were found to occur in partial linkage disequilibrium (LD) (\(r^2=0.61\)). Although ECs had markedly less representation of the 3’UTR rs1059234 mutant allele (T) and heterozygosity (CT) compared to progressors (T allele: 9.1% ECs vs. 25% progressors; CT genotype: 18.2% ECs vs. 42% progressors), this did not reach significance (p=0.11, OR=3.33; p=0.19, OR=3.49, respectively). Interestingly, HIV-1 controllers with <400 HIV-1 RNA copies/ml (<400 HICs) also had less representation of the CT genotype when compared to progressors (20% vs. 42%, respectively; p=0.11, OR=2.91).

In silico analysis of this 3’UTR SNP suggested that there are functional implications in terms of miRNA regulation, however when p21 mRNA expression was analysed with respect to this SNP, no effect was seen. The role of this 3’UTR SNP on p21 expression and/or function and HIV-1 control requires further investigation. The p21 exonic rs1801270 SNP showed no
difference in representation among the clinical phenotypic groups and no effect was seen on p21 mRNA expression.

When comparing HIV-1 controllers with >400 HIV-1 RNA copies/ml (>400 HICs) to progressors, the >400 HICs had significantly lower representation of the minor allele (A) of the miR-106b rs999885 SNP (p=0.04, OR=2.28). In addition, heterozygosity for this SNP (GA) was found in a much lower frequency in >400 HICs when compared to progressors (p=0.05; OR=2.56). Stratification of individuals according to their miR-106b rs999885 SNP genotype and p21 mRNA expression revealed the GA genotype to be associated with a trend to higher p21 mRNA expression (p=0.066). A role for the miR-106b rs999885 SNP in HIV-1 control in individuals with higher viraemia needs to be validated in larger cohorts.

Characterisation of the p21 regulatory regions, namely a region of the 5’UTR and the 3’UTR, identified 19 polymorphisms (18 SNPs and one indel) and 12 SNPs in the respective regions. A prevalent, previously uncharacterised 11-SNP haplotype (LD: r²=1) was detected in the p21 promoter region at a frequency of 39.42% in the HIV-1 controllers and 48.86% in the progressor cohort. In addition, a 2-SNP haplotype was identified and was found to be in moderate LD with the 11-SNP haplotype (r²=0.67). The ECs were found to have a trend of less representation of the 2-SNP haplotype minor allele when compared to progressors (p=0.08, OR=2.83). Other than the rs1059234 SNP, no other SNPs in the 3’UTR were differentially represented in any of our studied groups.

p21 mRNA expression analysis showed significant correlations between p21 mRNA expression and markers of disease progression (HIV-1 viral load: r=0.69, p<0.0001 and CD4+ T cell count: r=−0.53, p=0.0005). In our study, ECs and VCs had significantly lower p21 mRNA expression compared to progressors (p=0.002 and p=0.001, respectively). Furthermore, in our Black South African population (n=50), the p21 5’UTR rs733590 SNP CT and TT genotypes were not associated with higher p21 mRNA expression as has been shown in Caucasians. This, together with the absence of HLA-B*2705 in our Black South African population, points to host genetic differences as the likely contributors to the different results seen in our study with respect to p21 expression and HIV-1 control when compared to reported literature.

Future work with larger sample sizes and varied population groups will be highly informative in determining the role of p21 and natural control of HIV-1 in the Black South African population.