Low SKAP1 Levels Indicates a Potential New Mechanism in HIV Immunological Non-Responders

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BACKGROUND

Immunological non-responders (INR) are people living with HIV (PLHIV) who fail to restore CD4 T-cell counts under virally suppressive combination antiretroviral therapy (cART). INR risk for (non-AIDS) morbidity and mortality is increased and treatment options are lacking. We examined serum proteins and potential regulatory mechanisms to elucidate novel INR mechanisms. Src kinase associated phosphoprotein 1 (SKAP1) is an intracellular protein in lymphocytes that is important in lymphocyte activation and migration (through integrin LFA-1), and proliferation. SKAP1 knockout models showed impaired lymphocyte migration to the intestine and lymph nodes.

METHODS

Design and participants The 2000HIV study (clinicaltrials NTC03994835) is a cross sectional multi-center study of 1895 PLHIV in the Netherlands that consists of two cohorts: a discovery cohort and an independent validation cohort. Findings from the discovery cohort were checked in the validation cohort. INR were defined as CD4<350 after >2 years of adequate cART. Immunological responders (IR) were PLHIV with CD4>500.

Methods INR were compared to IR with regards to serum proteomics (Olink, 2368 proteins), intracellular SKAP1 levels in CD4 T-cells (western blot), whole blood DNA methylation (NanoDrop spectrophotometer). SKAP1 RNA transcription in CD4+ T-cells before and after HIV infection was compared in vitro. Single nucleotide polymorphisms (SNPs) predictive of SKAP1 serum levels were assessed, and their association with INR was examined.

Statistical analyses Serum proteomics and DNA methylation were compared using a linear model. Intracellular SKAP1 levels were analyzed using Mann-Whitney U test. For CD4 T-cell SKAP1 RNA expression trends are shown. SKAP1 serum SNP associations were investigated using a additive logistic regression model.

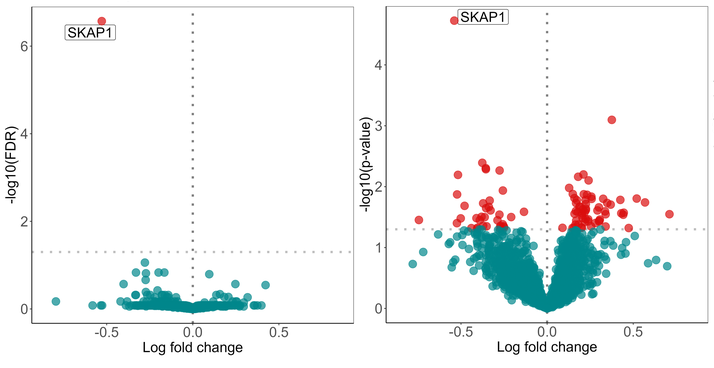
- Immunological non-responders have lower SKAP1 levels, which is epigenetically regulated
- HIV reduces SKAP1 transcription in-vitro
- SKAP1 plays a role in lymphocyte activation & proliferation, and is a potential INR drug target

RESULTS

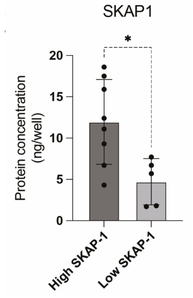
1.Demographics

	Discovery cohort			Validation cohort		
	INR n=62	IR n=1224	sig	INR n=26	IR n=243	sig
Current age	55	52	*	54	53	
Age at diagnosis	41	36	*	40	40	
CD4 Nadir	63	280	*	100	310	*
Time on cART	11.3	10.5	*	7.9	8.7	
History of non-AIDS malignancy	14.5%	3.4%	*	3.8%	6.2%	

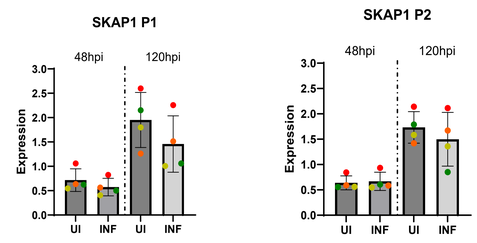
2. Serum proteomics: Low SKAP1 levels in INR



3. Intracellular levels confirm serum SKAP1

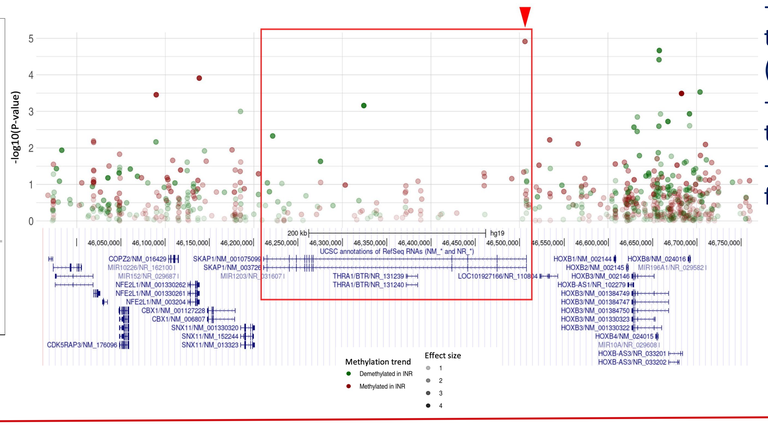


5. Reduced SKAP1 transcription in CD4+ T-cells after HIV infection in vitro

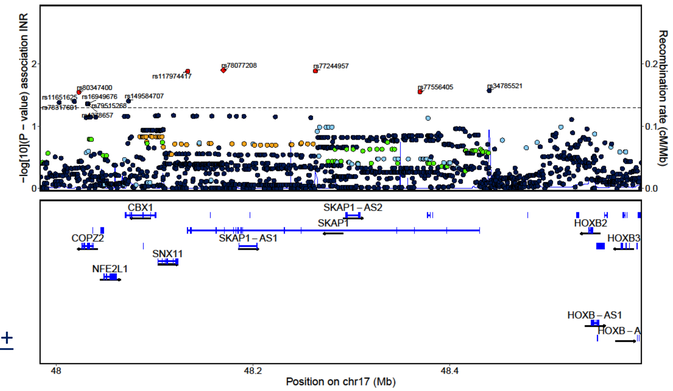


UI: HIV uninfected cells, INF: HIV infected cells, hpi: hours after infection

4. DNA methylation: SKAP1 promotor is hypermethylated in INR



6. SNPs associated with serum SKAP1 levels in SKAP1 region



No SKAP1 serum level SNPs were associated with INR

CONCLUSIONS

- Lower SKAP1 protein levels were found in serum and intracellularly in CD4+ T-cells in INR compared to IR
- INR have a hypermethylated SKAP1 promotor region, associated with decreased transcription
- SNPs in SKAP1 region did not relate to INR
- HIV infection decreases SKAP1 transcription in CD4+ T-cells in vitro (ns, trend)
- SKAP1 has never been described in the context of HIV or INR
- SKAP1 is a potential new drug target for INR.

ADDITIONAL INFORMATION

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www.2000HIV.com