P074

EVALUATION OF THE HLA-F VARIABILITY IN BRAZIL BY NEXT GENERATION SEQUENCING. Erick C. Castelli^a, Thálitta H.A. Lima^a, Renato V. Buttura^a, Michelle A. Paz^a, Iane O.P. Porto^a, Jaqueline Ramalho^a, Andreia S. Souza^a, Luciana C. Veiga-Castelli^b, Celso T. Mendes-Junior^c, Eduardo A. Donadi^b. ^aUNESP – Univ Estadual Paulista, Botucatu, SP, Brazil; ^bSchool of Medicine of Ribeirão Preto – USP, Ribeirão Preto, SP, Brazil; ^cFaculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Ribeirão Preto, SP, Brazil.

Aim: HLA-F is a non-classical HLA class I gene and is distinguished from its classical counterparts by low allelic polymorphism and distinctive expression patterns. The exact function of HLA-F remains unknown. It is believed that HLA-F has a tolerogenic function, based on its low variability and sequence conservation among primates. Currently, there is little information regarding the HLA-F allelic variation among human populations and available studies evaluated only a fraction of the HLA-F gene segment and/or searched for known alleles only. Here we evaluated the HLA-F variability (from –300 to the stop codon at position +2944, including introns) in 72 samples from the Southeastern Brazil, using next generation sequencing procedures.

Methods: HLA-F was amplified by PCR using generic primers. Sequencing libraries were obtained by using Nextera XT DNA (Illumina) and sequenced at the MiSeq platform (together with other HLA class I genes). Paired-end sequences were filtered and mapped by using a local software named HLA-mapper, which allows a reliable sequence mapping when several HLA genes are sequenced together. This procedure takes into account data from IMGT/HLA and 1000Genomes databases to properly assign each pair of sequences to the correct gene. Genotype calling was performed combining the Genome Analysis Toolkit (GATK) and local applications to get correct genotypes. Missing alleles and haplotypes were inferred by using the PHASE method, however, the known phase between variable sites (obtained by GATK) was considered.

Results: We found 39 variable sites arranged into 14 haplotypes. Of those, five were identical to a known haplotype described by the IMGT/HLA database, that includes F*01:01:01:01, F*01:01:01:08, F*01:01:03:01, F*01:01:03:03 and F*01:03:01:01, and they represent 75% of all haplotypes. The remaining haplotypes did present one or two nucleotide differences from a known IMGT/HLA haplotype. However, most of them were found at least twice in the present series. In addition, four new variable sites were detected.

Conclusion: We present here a strategy to evaluate HLA-F by NGS and the results do indicate that it is indeed conserved at the protein level, but the HLA-F worldwide haplotype variability might be higher than the one already described by IMGT/HLA.

P075

MONOCYTE RECRUITMENT TO HLA CLASS I ANTIBODY-ACTIVATED ENDOTHELIAL CELLS IS DEPENDENT UPON MTOR. Sahar Salehi, Elaine F. Reed. UCLA, Los Angeles, CA, United States.

Aim: Antibody-mediated rejection (AMR) persists as a major issue affecting solid organ transplants. The purpose of this study was to investigate the proximal signaling events that regulate mTOR-mediated leukocyte recruitment following HLA I stimulation in endothelial cells (EC).

Methods: For confocal microscopy experiments, EC were treated with rapamycin for 24 hours, stimulated with HLA antibodies, incubated for 1 h with sheep anti-ICAM-1 antibody, followed by 30 min incubation with secondary FITC-labeled goat anti-sheep IgG and then fixed. Cells were imaged by a Zeiss confocal microscope. EC were treated with mTOR inhibitors (RAD001 or Rapamycin) or U0126, PKC, Rho or ROCK inhibitors, then stimulated with HLA I antibodies. Phosphorylation of downstream effectors of the mTOR and Rho pathways were measured by Western blot. Recruitment of primary monocytes to EC was determined by static adherence assays.

Results: Using flow-based adherence assays, we previously discovered that mTOR inhibition impairs the ability of EC to support firm adhesion of tethered monocytes by affecting late stage ICAM-1 clustering, which has been attributed to Rho signaling. As demonstrated by confocal microscopy, this impairment was due to a decrease in association of actin stress fibers with ICAM-1, which disrupts ICAM-1 clustering. We determined by Western blot that mTOR operates upstream of RhoA, as inhibition of Rho did not disrupt phosphorylation of proteins downstream of the mTOR pathway, though inversely, mTOR inhibition blocked phosphorylation of downstream effectors of Rho. mTOR in turn regulates ERM phosphorylation, which is crucial for ICAM-1 clustering. RhoA pathway inhibition of EC blocked HLA-I antibody-induced recruitment of primary monocytes in static adherence assays.

Conclusion: Our findings connect mTOR-mediated class I signaling to cytoskeletal remodeling involving RhoA and ERM proteins, suggesting that mTOR inhibition reduces leukocyte recruitment to activated endothelium by impairing firm adhesion.