FUNCTIONAL AND "IN SILICO" ANALYSIS OF GENOMIC TRANSCRIPTIONAL SIGNATURE OF HUMAN SPERM SUBPOPULATIONS FROM ASTHENOZOOSPERMIC PATIENTS

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Abstract Body

Introduction: Human sperm contain unique RNAs that could be potential biomarkers of its function. In this study, gene expression from sperm subpopulations of healthy normozoospermic donors (D) and asthenozoospermic patients (P) were evaluated by RNA microarray technology with functional and bioinformatics analysis of genomic data.
Materials and Methods: Semen samples from 3 D and 3 P were used for gene expression analysis. For each sample, two sperm subpopulations were recovered by density gradient centrifugation: F1 (high motility; fraction used in ART) and F2 (low motility); total RNAs were isolated and hybridized to 12 Affymetrix microarrays (3PF1, 3PF2, 3DF1, 3DF2). Differentially expressed genes (DEG) were confirmed by qPCR and its functional analysis was performed with several bioinformatics approaches [FatiGO, REVIGO and Ingenuity Pathway Analysis (IPA)].
Results: The contrast PF1 *vs* DF1 showed 1831 DEG (59 up-regulated and 1772 down-regulated) and the contrast PF2 *vs* DF2 showed 211 DEG (17 up-regulated and 194 down-regulated) using a $|FC > 2|$ and $p < 0.01$. The directionality and magnitude of several DEG associated with sperm physiology were confirmed by RT-qPCR. The functional analysis of DEG from PF1 *vs* DF1 identified 507 biological processes (BP) overrepresented, particularly that involved in reproduction in multicellular organism. BPs like spermatid differentiation and development, cilium assembly and microtubule-based movement, fertilization and sperm-egg recognition were identified among those with high statistical significance. Functional analysis of DEG from PF2 *vs* DF2 did not generate any significant associated BP with reproduction. IPA of DEG from PF1 *vs* DF1 identifed Rac, integrin, actin cytoskeleton, NGF, ErbB and androgen signaling as those canonical pathways (CP) among the top down-regulated CPs, and predicted as decreased or inhibited within the asthenozoospermia bio-function.
Conclusion: Extensive and comprehensive functional analysis of genomic sperm data identified key genes involved in biological processes relevant to reproduction and sperm physiology. The description of key genes, as in this study, may identify individuals and sperm populations with higher fertilization potential, optimizing the evaluation of male fertility.

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