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Ilustração da Capa: Alexandre Takashi

SP.12.04 - CDC42 as an epigenetic regulator of ID4 in breast cancer

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Inhibitor of differentiation proteins 1, 2, 3 and 4 (ID1–4), are dominant negative regulators of the basic helix-loop helix family of transcription factors. In human tumors, an increased expression of ID proteins has been associated with reversion to an embryonic-like state, with loss of differentiation, high rates of proliferation, migration and neo-angiogenesis. In breast cancer, ID4 silencing by promoter hypermethylation is in ER+ (estrogen receptor) breast tumors and is associated with an increased risk of lymph node metastasis. However, in ER- breast tumors ID4 increased expression has been associated with an aggressive phenotype. Our group has shown that ID4 promoter hypomethylation is associated with the aggressive Triple Negative Breast cancer (TNBC) subtype. We demonstrated that ID4 hypomethylation is associated with BRCAness phenotype and downregulation of BRCA1 gene. We show that the overexpression of CDC42, a plasma membrane-associated small GTPase, induced ID4 promoter methylation in the TNBC cell line MDA-MDA231. Materials and Methods: MDA-MB321 cell lines were transfected with a CDC42-GFP or control-GFP vector. ID4 methylation was measured by droplet digital, MSP and MS-MLPA assay. Western Blot was perform to identify ID4 and BRCA1. We performed in silico analysis from TCGA. Discussion and Results: MS-MLPA assay revealed that ID4 methylation increased significantly in the Cdc42 transfected cells. WB revealed that ID4 protein expression decreased and BRCA1 expression increased in Cdc42 transfected cells. In silico studies revealed that Cdc42 expression was significantly associated with the expressions of: HDAC3, MBD2, MBD1 and YY1. Conclusion: CDC42 activates an epigenetic signaling pathway that induces ID4 methylation in MDA-MB231 cell lines. ID4 silencing after CDC42 transfection induces a less aggressive phenotype in TNBC cell lines.
Keywords: Methylation, ID4, Triple negative breast cancer

SP.12.05 - Genomic Analysis of Two Parasite Strains Isolated from a Fatal Case of Visceral Leishmaniasis

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Human Visceral Leishmaniasis (HVL) is a neglected disease caused by Leishmania parasites (two-host life cycle) transmitted by sand fly bites and can be lethal if untreated or treatment fails. Here, our aim was to analyze two clinical isolates obtained from a patient who died after disease's complication. The parasite strains were isolated from bone marrow aspirate (BM-s) and skin lesions (SL-s). First, molecular typing for Leishmania species identification was performed by PCR and MLEE, but the results were inconclusive. Then, we performed whole-genome sequencing analysis of both strains and a reference laboratory L. infantum strain (HU-UFS14) and compared them to reference genomes of Leishmania and other trypanosomatids. Illumina sequencing data (2x250; ~400Xcoverage) were quality checked and used for de novo assembly with ABySS producing ~4,500 contigs/scaffolds for BM-s/SL-s strains. The predicted proteome (over 10,000 proteins, using EMBOSS tool) and genome size (~54Mb) were much larger than expected for Leishmania genomes. A phylogenomic analysis based on ~4,500 orthologous protein sequences among 36 Trypanosomatidae genomes publicly available, showed that BM-s/SL-s strains were placed in a sister position to Crithidia fasciculata (one-host life cycle) and clustered apart from Leishmania clade. Corroborating this result, the whole-genome alignments performed by the MUMmer program, using L. infantum (32Mb) and *C. fasciculata* (41Mb) reference genomes, HU-UFS14 and BM-s/SL-s draft genomes, showed that BM-s/SL-s strains aligned to *C. fasciculata* whole genome with 92% identity, whereas only 1.9Mb aligned against *L. infantum* with 89% identity. *L.infantum*_x_HU-UFS14 whole-genome alignment presented 96% identity and *C. fasciculata*_x_HU-UFS14 presented small aligned region (~1.5Mb; 89% identity), as observed for BM-s/SL-s_x_*L.infantum*. Remarkably, 96% of whole-genome identity was found between BM-s and SL-s, showing that both belongs to the same species. Crithidia parasitize exclusively mosquitoes and is considered non-infective to humans. Importantly, these results show that unknown Crithidia-like parasites were isolated from a fatal HVL case.
Keywords: Visceral Leishmaniasis, genome alignment, Crithidia-like parasites
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