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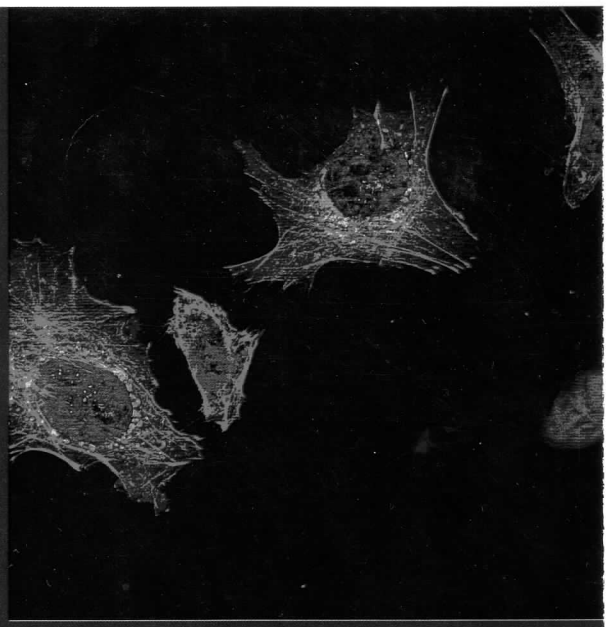

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Specific alterations of microRNA transcriptome and global network structure in colorectal carcinoma after cetuximab treatment

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The relationship between therapeutic response and modifications of miRNA transcriptome in Colorectal Cancer (CRC) remains unknown. We investigated this issue by profiling the expression of 667 miRNAs in two human CRC cell lines, one sensitive and the other resistant to cetuximab (Caco-2 and HCT-116, respectively) through TaqMan RT-PCR. Caco-2 and HCT-116 expressed different sets of miRNAs after treatment: specifically, 21 and 22 miRNAs were differentially expressed (DE) in Caco-2 or HCT-116, respectively (t-test, $p<0.01$). By testing the expression of DE miRNAs in CRC patients, we found that miR-146b-3p and miR-486-5p are more abundant in KRAS mutated samples respect to wild-type ones (Wilcoxon test, $p<0.05$). 67% of DE miRNAs were involved in cancer, including CRC, while 19 miRNA targets had been previously reported to be involved in the cetuximab pathway and CRC. We identified 25 TFs putatively controlling these miRNAs, 11 of which already reported to be involved in CRC. Based on these data, we suggest that the down regulation of let-7b and let-7e (targeting KRAS) and the up regulation of miR-17* (a CRC marker) could be considered as candidate molecular markers of cetuximab resistance. Global network functional analysis (based on miRNA targets) showed a significant overrepresentation of cancer-related biological processes and networks centered on critical nodes involved in EGFR internalization and ubiquitin-mediated degradation. The identification of miRNAs, whose expression is linked to the efficacy of therapy, should allow to predict the response of patients to treatment and possibly lead to a better understanding of the molecular mechanisms of drug response.

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A molecular Systems Medicine approach to Diabetes Mellitus pinpoints the molecular bases of the higher resistance to cytokine-induced apoptosis of mammalian pancreas alpha cells respect to beta cells

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Background Diabetes Mellitus (DM) is a pathogenetically complex and heterogeneous systemic syndrome that afflicts millions of people worldwide. Seeking to gain a molecular systems view on its etiology, we exploited an in vitro model to investigate the global involvement of the Apoptotic Machinery (AM) in DM onset and progression. Methods Through high-throughput technology, we studied the alterations of AM transcriptome and AM critical protein nodes in two mouse cell lines from pancreas salpha- and beta-cells (alphaTC1 and betaTC1, respectively), after treating both with a cocktail of cytokines for a time course of 24 h, 48 h, 72 h: according to a general consensus, this model appropriately reproduces in vitro the inflammatory environment that through different mechanisms is involved in the pathogenesis of both T1DM and T2DM. AM protein- encoding genes, markedly over-expressed or down-regulated and conserved between the mouse and Homo sapiens, were ranked through functional (Fisher inverse chi-square test) and protein-protein interaction (Wilcoxon matched-pairs signed-ranks test) prioritization methods, as well as Genome Wide Association (GWA) and epidemiological data. MIR genes, targeting AM protein encoding genes, were ranked based on their dysregulation, that of their targets, and genome position. Results Overall, 31 of 92 AM protein-encoding genes significantly (more than threefold) varied their expression with respect to matched controls. In alphaTC1 cells, NOS2 was highly induced, whereas neither Ser 20-phosphorylated P53, Tyr 705-phosphorylated STAT3, nor the death receptor TNFRSF10B changed their levels. In betaTC1 cells, we detected the increase of proapoptotic proteins ATF3, BNIP3, NOS2, Ser 20-phosphorylated P53, TNFRSF10B, and the decrease of antiapoptoticTyr 705- phosphorylated STAT3. The AM molecular profile in alpha cells, including P53 and STAT3 pathways, was comparable to controls also after treatment with regulatory AM networks in cytokines. On the contrary, reconstruction of beta cells demonstrated the activation cytokine-treated of proapoptotic P53 and of both canonical and alternative NFkB pathways, coupled to inactivation of antiapoptotic STAT3 pathway. Conclusions Our molecular characterization of pancreas alpha cells, to date poorly studied, has allowed to pinpoint the molecular bases of their higher resistance to cytokine- induced apoptosis respect to beta cells. Based on our experimental data and computational analysis, we prioritized AM protein-encoding genes DDIT3, MAP3K14, NFKB1, NFKBIA, NFKBIB, NFKB2, RELA, STAT3 and AM MIR genes MIR16, MIR124, MIR199a, MIR497 as DM candidate genes: the previous identification of some of these through functional, GWA, epidemiologic studies strengthens our proposal. Bibliography Di Pietro C, Ragusa M, Barbagallo D et al: The apoptotic machinery as a biological complex system: analysis of its omics and evolution, identification of candidate genes for fourteen major types of cancer, and experimental validation in CML and neuroblastoma. BMC Med Genomics 2009, 2: 20. D Barbagallo, S Piro, M Ragusa et al: A molecular Systems Medicine approach to Diabetes Mellitus pinpoints the molecular bases of the higher resistance to cytokine-induced apoptosis of mammalian pancreas alpha cells respect to beta cells. BMC Genomics, inviato per la pubblicazione.

Molecular profiling of human oocytes after vitrification strongly suggests that they are biologically comparable to freshly isolated gametes

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Oocytes cryopreservation is a helpful fertility preservation technique for women at risk of loosing their ovarian functions following disease, surgery or chemotherapy. Moreover, avoiding embryo cryopreservation would solve religious, ethical and legal problems, connected to the laws that actually regulate medically assisted reproduction in various countries. There are two major techniques for cryopreservation: slow freezing and vitrification. Many published studies have compared frozen-thawed human oocytes, either after slow freezing, or vitrification with fresh collected ones and they have analyzed their biologic behaviour as well as more specific structural cellular features. However, there are no published data on the molecular profile of oocytes after cryopreservation. To assess the effects of vitrification on the biologic quality of oocytes, we compared the expression profile of mRNAs in single vitrificated-thawed oocytes with that of freshly collected oocytes without cryopreservation. We report the expression analysis of eight different genes: three perform housekeeping functions, since they encode proteins involved in the basic cellular functions and are constantly expressed in all human cells [HPRT, GAPDH, CYCLOPHILIN)]; the other five genes encode proteins essential for oocyte development and specific functions [BMP15, GDF9, FIGLA, OCT4, TAF4B]. The transcripts chosen for our analysis encode proteins that are essential for mature oocytes, due to their role in gamete viability and in follicular and embryo development: accordingly, they represent excellent molecular markers of oocyte quality. Human oocytes were collected from an IVF Centre (Servizio di PMA/ Azienda Ospedaliera Cannizzaro, Catania, Italy), after informed consent for the use of supernumerary ones. A total of 25 metaphase II (MII) oocytes were collected from 5 different women, whose primary infertility was due to a male factor: this excluded pathologies that could influence oocyte quality. For each patient, we collected 2 fresh and 3 vitrificated oocytes, choosing those with optimal morphology, for a total of 10 fresh and 15 vitrificated oocytes. Our data clearly show that the expression profile of the eight genes, chosen as biomarkers, did not change between fresh and vitrificated oocytes . To statistically validate our results, we compared the Ct value, normalized to HPRT (DeltaCt), in fresh and vitrificated oocytes by using the independent Student's t-test and demonstrated that there are no significant variations between fresh and vitrificated-thawed oocytes. Our molecular data, together with published results on oocyte survival, oocyte fertilization and pregnancy rates, confirm that vitrification might be very helpful for preserving women fertility. REFERENCES Purrello M, Di Pietro et al. Genes for human general transcription initiation factors TFIIIB, TFIIIB-associated proteins, TFIIIC2 and PTF/SNAPC: functional and positional candidates for tumour predisposition or inherited genetic diseases? ONCOGENE. 2001 Aug 9;20(35):4877-83. C Di Pietro et al Molecular profiling of human oocytes after vitrification strongly suggests that they are biologically comparable to freshly isolated gametes. FERTILITY AND STERILITY, (e-pub ahead of print on 9 June 2010).