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Enterovirus genome and infectivity in peripheral blood leukocytes of children at the clinical onset of type 1 diabetes

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Background and aims: Enterovirus (EV) infections are regarded as prominent environmental factors in the early stages of type 1 diabetes (T1D). On the day of clinical diagnosis of T1D, EV genome and infectivity were searched for in total peripheral blood leukocytes (PBL) of pediatric patients.
Materials and methods: This observational study was approved by the local Ethics Committee. PBL were obtained from 114 children on the day of T1D diagnosis at two Pediatric Endocrinology Centers in Italy (median age 9.0 yrs; range 2-16 yrs). EV-susceptible cell lines (RD, HeLa, AV3, CaCo) were co-cultured with the patients` PBL. Primers covering the 5`UTR, VP4, and 3D genome regions of EV of the A, B, C, and D species (≥100 types) were used in highly sensitive RT-PCR assays that were run both on plasma and tissue culture medium from cell cultures exposed to patients` PBL. Expression of viral capsid proteins was evaluated in infected cell cultures using antiviral mAbs directed to the capsid protein VP1. Routine methods were used to measure levels of blood glucose, HbA1c, C-peptide (time 0 and 6 min after glucagon stimulation), diabetes related auto-Abs (GAD65, IA2, ZnT8, IAA), and - one year after diagnosis - the insulin requirement (IU/Kg/day). Immunoassays were used to quantify cytokines released by cultured cells. On the day of diagnosis, EV genome and infectivity were also searched for in consenting family members of 20 children.
Results: EV genome fragments and infectivity were detected in PBL of 90/114 (79%) children, versus 3/75 (4.0%) matched non-diabetic controls. Preliminary EV identification based on sequences of the 3Dpol genome region showed that EV of the B species were predominant (58% of positives). Viruses of the A, C, and D species were also detected. Tests on cell lines exposed to patients PBL confirmed the intracellular production of viral capsid antigen (immunofluorescence and WB). As compared to cell cultures exposed to controls' PBL, cell lines that had been exposed to patients' PBL released enhanced levels of the MCP1 chemokine. At the time of diagnosis, EV-positive patients had significantly higher levels of glucose and HbA1c as compared to EV-negative diabetic children. EV-positive children had also significantly reduced levels of glucagon-stimulated C-peptide. One year post-diagnosis, the insulin requirement was not different between the two groups. In a consenting cohort, EV genome and infectivity were found in blood of 18/20 (90%) diabetic children, 12/17 (70%) non-diabetic asymptomatic siblings, and 17/28 (61%) asymptomatic parents. Virus-positive members of each family shared the same EV species.
Conclusion: a) Detection of EV in blood represents a frequent biomarker of early stage T1D; b) EV of different species can be detected in newly-diagnosed patients (i.e., different EV species can associate with the early clinical stages of T1D); c) EV activity is expressed in human cell lines both as production of viral antigen and enhanced release of an inflammatory chemokine; d) EV genome and infectivity can be found both in newly diagnosed children and in their non-diabetic family members. This observation indicates that EV infections are spreading within the family at the early clinical stages of T1D.
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OP 16 Mechanisms of insulin action

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Aerobic glycolysis is a major regulator of Akt activity

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Background and aims: Tumour cells exhibit inordinately high levels of aerobic glycolysis, a phenomenon known as the Warburg effect. While the functional relevance of this effect is not known it is partly driven by upregulation of the enzyme PFKFB3 (6-Phospho-2-Fructo Kinase/Fructose-2,6-Bisphosphatase), which controls the synthesis of fructose-2,6-bisphosphate, an allosteric activator of glycolysis. Intriguingly, adipocytes exhibit the highest expression of PFKFB3 among most mammalian cell types. In view of the important role of the fat cell in nutrient sensing we wondered if PFKFB3 might play a role in this sensory mechanism. Consistent with previous studies we showed that fat cells apportioned the majority of their glucose uptake toward glycolysis. To test the role of PFKFB3 as a fuel sensor in adipocytes we examined the consequences of perturbing its activity and/or expression levels on glucose metabolism and insulin signalling.
Materials and methods: Insulin signalling was examined by immunoblot. Glucose uptake was measured using [^{3}H]2-deoxy-glucose. GLUT4 translocation was measured by cell surface labelling of HA-GLUT4 expressing cells. Lactate production was measured by extracellular flux analyser. The specific inhibitor of PFKFB3, 3PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one), was used to inhibit glycolysis. Overexpression of PFKFB3 and the bisphosphatase domain of PFKFB2 (BP-2) was used to increase or inhibit glycolysis, respectively. 3T3-L1 cells were electroporated with siRNA targeting the PFKFB3 sequence.
Results: Incubation of adipocytes with the PFKFB3 inhibitor 3PO led to a dose dependent decrease in insulin-stimulated glucose uptake and GLUT4 translocation. 100 μM 3PO inhibited glucose uptake and GLUT4 translocation by 70% ($p<0.01$) and 50% (p40% at sub-maximal dose (10 μM). To further investigate the relationship between PFKFB3 activity and Akt signalling we examined the effect of over-expression or siRNA knock down of PFKFB3, or overexpression of the PFKFB3 antagonist BP-2, in HEK-293 cells. Knock down of PFKFB3 or BP-2 overexpression decreased insulin-stimulated Akt phosphorylation at Ser473 by 50% while PFKFB3 overexpression *potentiated insulin-stimulated Akt phosphorylation* increasing it 4 fold.
Conclusion: These studies reveal a novel mechanism for controlling Akt activity involving glucose flux via the glycolytic pathway. This pathway has potential implications for both cancer, where Akt activity may be driven by over stimulation of glycolysis, and diabetes where impaired glucose uptake may compromise Akt activity. The 'feedforward' mechanism by which insulin stimulation increases glycolysis is well described. Our study reveals a 'feedback' mechanism by which a defect in glycolysis decreases growth factor signalling through Akt.

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Upregulation of miRNA-143 expression by secretory products from epicardial adipose tissue from patients with type 2 diabetes abrogates insulin action in cardiomyocytes

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Background and aims: Cardiac dysfunction and myocardial insulin resistance are common in patients with type 2 diabetes. We have recently found that conditioned media (CM) generated from epicardial adipose tissue from patients with type 2 diabetes (CM-EAT-T2D) abrogate insulin action in primary rat cardiomyocytes. This was not observed in cardiomyocytes exposed to CM-EAT from patients without type 2 diabetes (CM-EAT-ND) and from subcutaneous (SAT) and pericardial adipose tissue (PAT) from the same patients. In this study, we examined whether the induction of insulin resistance can be ascribed to alterations in miRNA expression induced by CM-EAT-T2D.
Materials and methods: Biopsies from EAT, PAT and intrathoracal SAT were collected from males of Caucasian origin undergoing open heart surgery.