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Downregulation of A_1 and A_{2B} adenosine receptors in human trisomy 21 mesenchymal cells from first-trimester chorionic villi

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ABSTRACT

Human reproduction is complex and prone to failure. Though causes of miscarriage remain unclear, adenosine, a proangiogenic nucleoside, may help determine pregnancy outcome. Although adenosine receptor (AR) expression has been characterized in euploid pregnancies, no information is available for aneuploidies, which, as prone to spontaneous abortion (SA), are a potential model for shedding light on the mechanism regulating this event. AR expression was investigated in 71 first-trimester chorionic villi (CV) samples and cultured mesenchymal cells (MC) from euploid and TR21 pregnancies, one of the most frequent autosomal aneuploidy, with a view to elucidating their potential role in the modulation of vascular endothelial growth factor (VEGF) and nitric oxide (NO). Compared to euploid cells, reduced A_1 and A_{2B} expression was revealed in TR21 CV and MCs. The non-selective adenosine agonist 5′-*N*-ethylcarboxamidoadenosine (NECA) increased NO, by activating, predominantly, A_1AR and A_{2A}AR through a molecular pathway involving hypoxia-inducible-factor-1 (HIF-1α), and increased VEGF, mainly through A_{2B}. In conclusion the adenosine transduction cascade appears to be disturbed in TR21 through reduced expression of A_{2B} and A_1ARs. These anomalies may be implicated in complications such as fetal growth restriction, malformation and/or SA, well known features of aneuploid pregnancies. Therefore A_1 and A_{2B}ARs could be potential biomarkers able to provide an early indication of SA risk and their stimulation may turn out to improve fetoplacental perfusion by increasing NO and VEGF.

1. Introduction

15% of human pregnancies are known to end in spontaneous abortion (SA) before 12 weeks of gestation, and immunity, angiogenesis and apoptosis-related genes have all been implicated. In aneuploidy, however, the reported percentage of SA is much higher [1]. One possible reason could be that the chromosomal abnormality itself leads to miscarriage, but if this is the case, the pathogenic mechanism is still unknown.

It has also been suggested that the causes of SA in aneuploidy are no different to those in euploidy, with the increased frequency in the former perhaps being ascribable to a genetically-determined imbalance in the mediators of placental perfusion and uterine contraction [1]. In this scenario, mediators such as endothelial growth factor (VEGF) and nitric oxide (NO) may be involved; indeed, a critical role has been reported for both in placental angiogenesis [2,3]. During gestation, angiogenesis occurs extensively in the placenta and villi to supply the fetus with oxygen and nutrition. This vascular development during embryonic and fetal growth in utero is triggered by hypoxia, a condition that is also known to increase the levels of adenosine (Ado) [4]. This important hormone is locally released from metabolically active cells, or generated extracellularly by the degradation of ATP. Acting through its receptor (AR) subtypes A_1, A_{2A}, A_{2B} and A_3, this nucleoside has been shown to regulate a wide variety of physiological processes, including angiogenesis in hypoxic tissues [5]. In particular, Ado plays an important role in the regulation of VEGF from placental villi in hypoxic conditions, and it also increases NO

Abbreviations: CV, chorionic villi; DPCPX, 1,3-dipropyl-8-cyclopentyl-xanthine; MC, mesenchymal cells; MRE 2029-F20, *N*-benzo[1,3]dioxol-5-yl-2-[5-(1,3-dipropyl-2,6-dioxo-2,3,6,7-tetrahydro-1 H-purin-8-yl)-1-methyl-1 H-pyrazol-3-yl-oxy]-acetamide]; MRE 3008-F20, 5-*N*-(4-methoxyphenylcarbamoyl)-amino-8-propyl-2-(2-furyl)-pyrazolo[4,3e]-,2,4triazolo[1,5c]pyrimidine; NECA, 5′-*N*-Ethylcarboxamidoadenosine; PSB36, 1-Butyl-8-(hexahydro-2,5-methanopentalen-3a(1 *H*)-yl)-3,7-dihydro-3-(3-hydroxypropyl)-1 *H*-purine-2,6-dione; PSB603, 8-[4-[4-(4-Chlorophenzyl)piperazide-1-sulfonyl)phenyl]]-1-propylxanthine; SCH442416, 2-(2-Furanyl)-7-[3-(4-methoxyphenyl)propyl]-7 *H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine; ZM, 241385 (4-(2-[7-amino-2-(2-furyl)-[1,2,4]triazolo-[2,32][1,3,6]triazinyl-amino]ethyl)-phenol)

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synthesis in the fetoplacental endothelium; it is therefore considered to be a major factor in maintaining normal fetoplacental function [6,7].

Although AR expression has been characterized in human placenta from normal pregnancies, no data are as yet available concerning expression and signaling cascades triggered by ARs in aneuploidies [8,9]. Nevertheless, as previously mentioned, aneuploidies very often end in SA, making them a good experimental model for potentially shedding light on the mechanism regulating this event. The aim of this study was therefore to investigate the expression of ARs in first-trimester chorionic villi (CV) and isolated mesenchymal cells (MC) from both euploid (E) and trisomy (TR) 21 pregnancies, one of the most frequent autosomal aneuploidy; viable cells, namely those obtained via routine chorionic villus sampling, rather than spontaneous abortus tissue, were chosen, as any alteration of ARs in the latter could be a consequence rather than the cause of miscarriage. The rationale behind the study was that elucidating the role of Ado in the modulation of important proangiogenic molecules like VEGF and NO in aneuploid pregnancies may also shed light on the proteins and pathways involved in SA in euploid pregnancy.

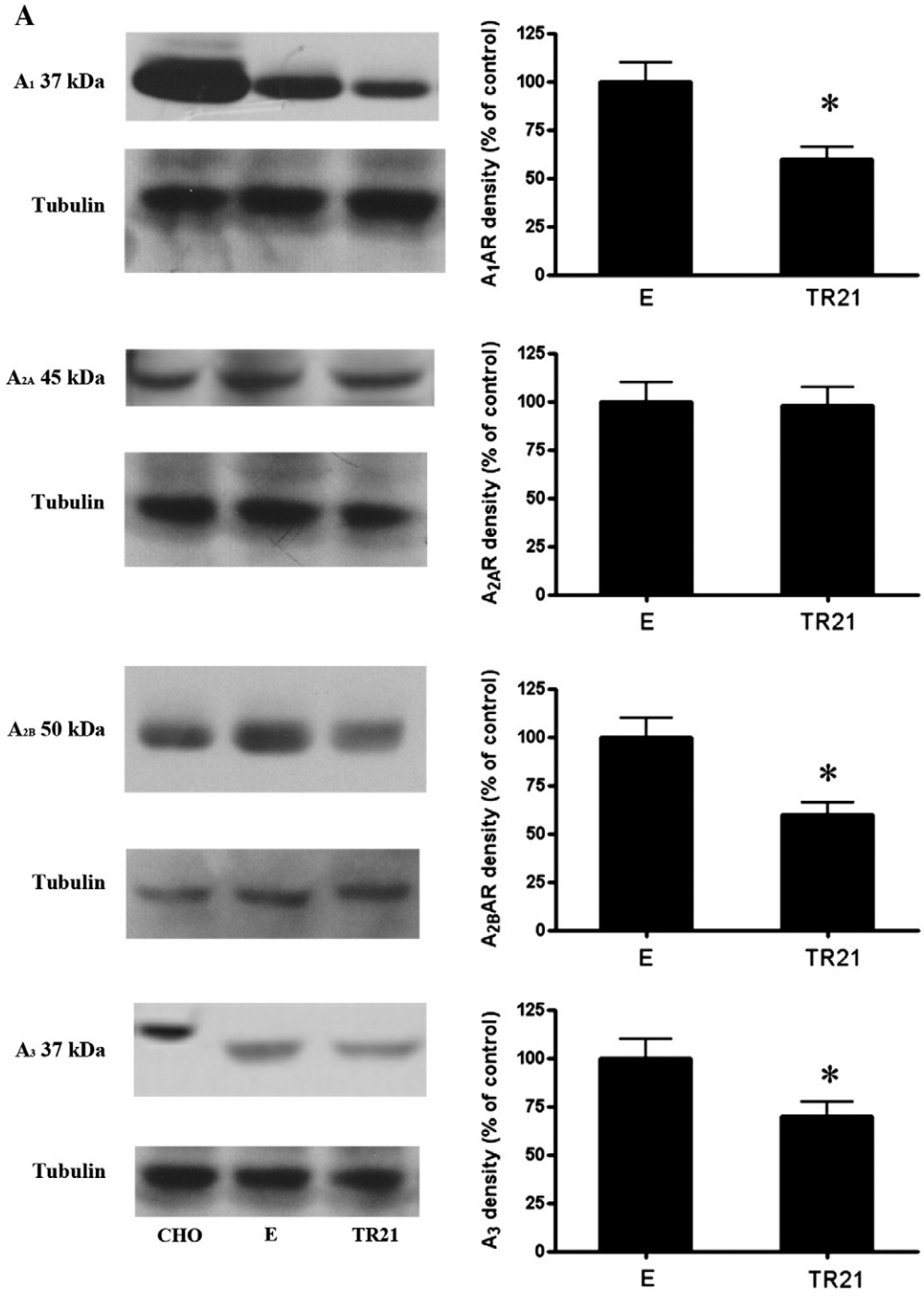

Fig. 1. Expression levels of A_1, A_{2A}, A_{2B}, A_3AR proteins in E and TR21 CV. Representative Western blot analyses of ARs in CV biopsies from women with E (line 2) and TR (line 3) pregnancies at 12 weeks of gestation. CHO cells transfected with the different ARs were loaded as positive control (line 1). Histograms represent % decrease with respect to E pregnancies. Densitometric quantification of Western blots is the mean ± SE values (N = 4 for each group) *P<0.01 vs E CV. (A); Saturation curves of [^{3}H]DPCPX, [^{3}H]ZM 241385, [^{3}H]MRE 2029 F20 and [^{3}H]MRE 3008 F20 binding to A_1, A_{2A}, A_{2B}, A_3ARs in CV biopsies from E (N = 13) and TR21 pregnancies (N = 10) (B).Specific (■) and nonspecific equilibrium binding (▲) were determined as described in the methods. Each value represents the mean ± SEM of experiments performed in duplicate.