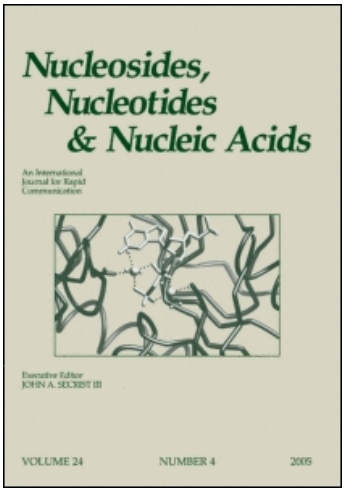

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Expression of Human Ecto 5' Nucleotidase in Pig Endothelial Cells and Its Implication for Adenosine Production and Xenotransplantation

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EXPRESSION OF HUMAN ECTO 5′ NUCLEOTIDASE IN PIG ENDOTHELIAL CELLS AND ITS IMPLICATION FOR ADENOSINE PRODUCTION AND XENOTRANSPLANTATION

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▫ *Human endothelial activity of ecto-5′-nucleotidase (E5′N) is several times higher than in pig endothelial cells. This may have implication for xenotransplantation due to the role this enzyme plays in conversion of pro-inflammatory and pro-aggreggatory nucleotides into anti-inflammatory and anti-aggregatory adenosine. We have shown in this study that human E5′N can be functionally expressed in pig endothelial cells leading to increased adenosine production from both extracellular AMP and ATP. We suggest that E5′N expression in transgenic pigs for xenotransplantation may help to prolong graft survival.*

Keywords Ecto-5′-Nucleotidase, CD73, Adenosine, ATP, Nucleotides, Xenotransplantation

INTRODUCTION

Ecto 5′ nucleotidase (E5′N) or CD73 is an endothelial surface enzyme involved in extracellular nucleotide breakdown leading to the formation of anti-inflammatory and immunosuppressive adenosine.[1–4] Expression of E5′N in pig cells has been found to be one order of magnitude lower than in human endothelium.[5] Thus, human EC has a significantly higher ability than porcine EC to catalyze the extracellular conversion of AMP to adenosine. This is particularly

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important for xenotransplantation where metabolism of extracellular nucleotides into adenosine plays a crucial role in interaction of endothelial cells with inflammatory cells and platelets.

METHODS

The pig intestinal endothelial cell line (PIEC) was transfected using Effectene with human endothelial cDNA cloned into pCDNA3.1 bearing the neomycin resistance element and cell clones with the highest activity were selected using serial dilutions. Cells used were >95% human CD73 positive by flow cytometry. E5′N activity was measured at 37°C under conditions optimal for extracellular but not for cytosolic isoform of 5′-nucleotidase.[6] Before assay cells were lysed with extraction buffer as described in detail previously,[6] rates of degradation of AMP or ATP in intact cells were measured by HPLC analysis[7] of cell media following incubation of confluent endothelial cells with 50 μM AMP or ATP for different times. Protein was measured using Bradford assay.

RESULTS AND DISCUSSION

Activity of E5′N in lysates of transfected and nontransfected PIEC compared to human umbilical vein EC (HUVEC) is presented in Table 1. This data indicate that transfection caused more than 1000-fold increase in E5′N activity. These results confirm that pig activity of E5′N is much lower than human.

Rate of adenosine production in intact cells from exogenous AMP was 177 ± 9.6 nmol/mg protein/min in E5′N transfected cells, while in nontransfected cells the calculated rate was 0.67 ± 0.06 nmol/mg protein/min. This lower activity found in intact cells could be a consequence of limited access of substrate to enzyme present on side of the cell attached to culture flask. Expression of human E5′N in pig cells caused increase in the rate of adenosine formation from extracellular ATP in the intact cells. The calculated rate of adenosine production by the nontransfected cells was 0.296 ± 0.031 nmol/mg protein/min while it increased to 0.642 ± 0.050 nmol/mg protein/min in E5′N-transfected cells. We conclude that human E5′N could be functionally expressed on the surface of pig endothelial cells. Increased production of adenosine not only from AMP but also from ATP demonstrates that E5′N activity is the rate limiting step for

TABLE 1 Ecto-5′Nucleotidase Activity in Cell Lysates of Nontransfected and Human E5′N-Transfected Pig Endothelial Cells and HUVEC

Ecto-5′-nucleotidase activity (nmol/mg protein/min)			
Non-transfected PIEC	Transfected PIEC	PAEC	HUVEC
0.68 ± 0.07	1013 ± 293	2.79 ± 0.36	35.7 ± 2.5

Values are mean ± S.D., $n = 5–8$.

extracellular adenine nucleotide breakdown and adenosine production. We propose that E5′N overexpression in transgenic pigs would be a successful strategy for xenotransplantation.

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