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# Impact of Human CD31 Transgenic Modulation in Xenotransplantation on Neutrophil Extracellular Traps

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**Introduction:** Sustained systemic inflammation in xenograft recipients (SIXR) remains a barrier to the successful application of xenotransplantation. Neutrophil Extracellular Traps (NETs) could be a mechanism of SIXR; however, comprehensive studies on NETs in Xenotransplantation remain scarce due to the technical hurdles of NETs observation. This study aims to investigate the presence and impact of NETs in a Xenograft setting and explore the modulatory effect of human CD31 transgenic overexpression on NETs.

**Methods:** NETs were studied in an ex-vivo xenotransplantation mimicking system using human clinical samples. Isolated human neutrophils were cocultured with various cell lines, including the wild-type porcine aortic endothelial cell line (PAEC), the hCD31 overexpressed PAEC, and the human aortic endothelial cell line (HAEC). NETs were assessed and quantified using label-free imaging by holotomography. The supernatant of coculture was used to measure cytokines and histone-DNA complexes.

**Results:** During coculture of human neutrophils with porcine-derived cell lines, the typical formation of NETs was observed, which showed typical autophagic vacuoles followed by chromatin swelling. In contrast, there was no sign of typical cellular changes of NETs in human neutrophils cocultured with HAEC. Intriguingly, the expression of the human CD31 transgene exerted a suppressive influence on NETs, resulting in altered dynamics of NET formation. The suppressive effect of hCD31 overexpressed PAEC on NETs was corroborated by the DNA-histone complex and human IL-8, measured by ELISA from the coculture supernatant. DNA-histone complex was reduced in the supernatant from hCD31 overexpressed PAEC coculture ($1.062 \pm 0.092$ vs $0.477 \pm 0.146$, $p<0.005$, Figure 1B). Human IL-8 was also reduced in the supernatant from hCD31 overexpressed PAEC coculture ($9352 \pm 1161$ vs $4210 \pm 670.4$, $p<0.0005$, Figure 1C).

**Conclusion:** We provide the evidence that human CD31 overexpression in porcine cell show a suppressive effect on NETs formation, which might be the therapeutic target of SIXR.