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Molecular Mechanisms of Endothelial Progenitor Cell Attachment and Retention on Cholesterol Modified Polyurethane Heart Valve Leaflets

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Objectives:

We previously reported retention of autologous ovine endothelial precursor cells (EPCs) seeded on the surface of implanted pulmonary valve leaflets composed of cholesterol modified polyurethane (Chol-PEL). CD 47, also known as integrin associated protein, forms a cholesterol dependent complex with $\alpha_v\beta_3$ integrin and Gi protein that enhances cellular attachment. In these experiments we investigated the molecular components that facilitate EPC binding to Chol-PEL surfaces. Our working hypothesis is that the CD 47 signaling pathway is an important contributor to EPC attachment and retention on Chol-PEL heart valve leaflets.

Method:

Human EPC lysates were immunoprecipitated with immunoaffinity bead conjugated anti-CD47 antibody, and the associated components of the CD47 molecular complex were identified using standard Western blotting techniques. To assess the contribution of the CD47 complex to EPC attachment, PEL-Chol or control surfaces were seeded with suspended EPCs in the presence of function blocking antibodies. At timed endpoints, the films were washed, fixed with 4% paraformaldehyde, and the attached cells were quantified.

Results:

Western blotting of CD47 immunoprecipitated complexes detected both $\alpha_v\beta_3$ integrin and Gi protein. The presence of either anti-CD47 or anti- $\alpha_v\beta_3$ antibodies significantly ($p<0.05$) reduced the number of adherent EPCs on Chol-PEL surfaces.

Conclusions:

These data are the first demonstration of the presence of the CD47 signaling complex in EPCs. In addition these results show a role for the CD47 signaling complex in EPC attachment to Chol-PEL surfaces, that explains in part the mechanistic basis for the successful adhesion of seeded EPCs on Chol-PEL noted in sheep circulatory explants.