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Visualization of Phosphorylated Platelet-derived Growth Factor Receptors (pPDGFR) in Mouse Leucocytes Using Quantum Dots

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Platelet-derived growth factor receptor (PDGFR) is expressed in blood leucocytes [1] and the ligand, PDGF, is stored in great abundance in alpha granules of platelets. The objective of this study is to identify the cell types that express PDGFR and determine the phosphorylation status of the receptor in circulating peripheral blood leucocytes of athymic nude mice by correlative sequential microscopy of samples immunolabeled with quantum dots (Qdots)[2] using wide field and confocal fluorescence microscopy and transmission electron microscopy (TEM).

Leucocytes isolated subsequent to erythrocyte lysis of blood drawn in EDTA-containing tubes were fixed in 1% paraformaldehyde in PBS for 20 min. Cytospin preparations were immersed in cold acetone for 20 min and rinsed with PBS. Immunocytochemistry was performed by sequential incubation of the cells in (a) blocking buffer containing anti-Fc receptor antibodies, (b) rabbit anti-pPDGFR , (c) Qdot 655 conjugated anti-rabbit IgG, and (d) counterstained with either Hoechst 33342 or Sytox green. All antibody incubations were for 1 h at ambient temperature with several washes in PBS between incubations. The samples were mounted in media containing glycerol, PBS, and propyl gallate. Fluorescence was examined using both wide field fluorescence microscopy and confocal microscopy. Controls included incubation of samples with (a) Qdot-labeled secondary antibody alone, (b) Qdot labeled anti-phosphotyrosine antibody, and (c) incubation of duplicate samples using Cy5-conjugated secondary antibody. For TEM, immunolabeled samples were placed in a fixative containing 2% paraformaldehyde and 3% glutaraldehyde for 1h, osmicated for 1h, and stained en bloc with uranyl acetate for 1 hr before embedding in Spurr's low viscosity medium. Polymerized blocks were cut and examined directly without lead staining in a JEOL 1010 TEM at an accelerating voltage of 80 kV.

Leucocytes from normal mice showed heterogeneous labeling for pPDGFR, and neutrophils, identified by their multi-lobed nuclei, showed the most cytoplasmic label (Fig.1). A subpopulation of leucocytes was negative and served as an internal labeling control. A similar pattern and frequency of labeled cells was observed for leucocytes immunolabeled with Cy5. Labeling with Qdot-anti-phosphotyrosine confirmed labeling of phosphorylated tyrosine in leucocytes. TEM of the samples tested for reactivity to anti-pPDGFR showed that the electron-dense cores of the Qdots were identifiable and were observed mainly in neutrophils and less so in other leucocytes (Fig.2). Qdots were seen at the plasma membrane but the majority were in the cytoplasm and often in close proximity to cytoplasmic granules (Fig 3.) We conclude that Qdot-labeled antibodies are excellent for immunocytochemistry, not only because of the non-fading characteristic of this label, but also the feasibility of corroborative microscopy by TEM.

References:

[1] C-H. Heldin and B.Westermark, Physiol. Rev. 79 (1999) 1283.
[2] J.K. Jaiswal et al., Nature Methods, 2004) 73.

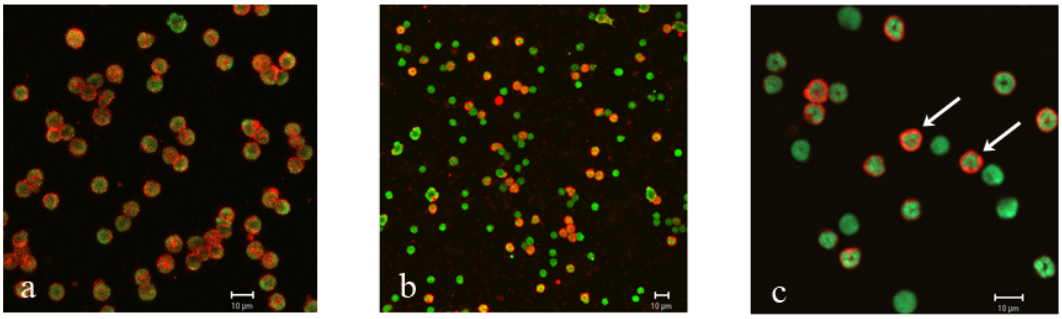

Fig. 1. Immunolabeling with Qdots for PDGFR (a), pPDGFR (b) and phosphotyrosine (c). Nearly all cells expressed PDGFR but only a fraction expressed pPDGFR or phosphotyrosine. Arrows show cells positive for phosphotyrosine.

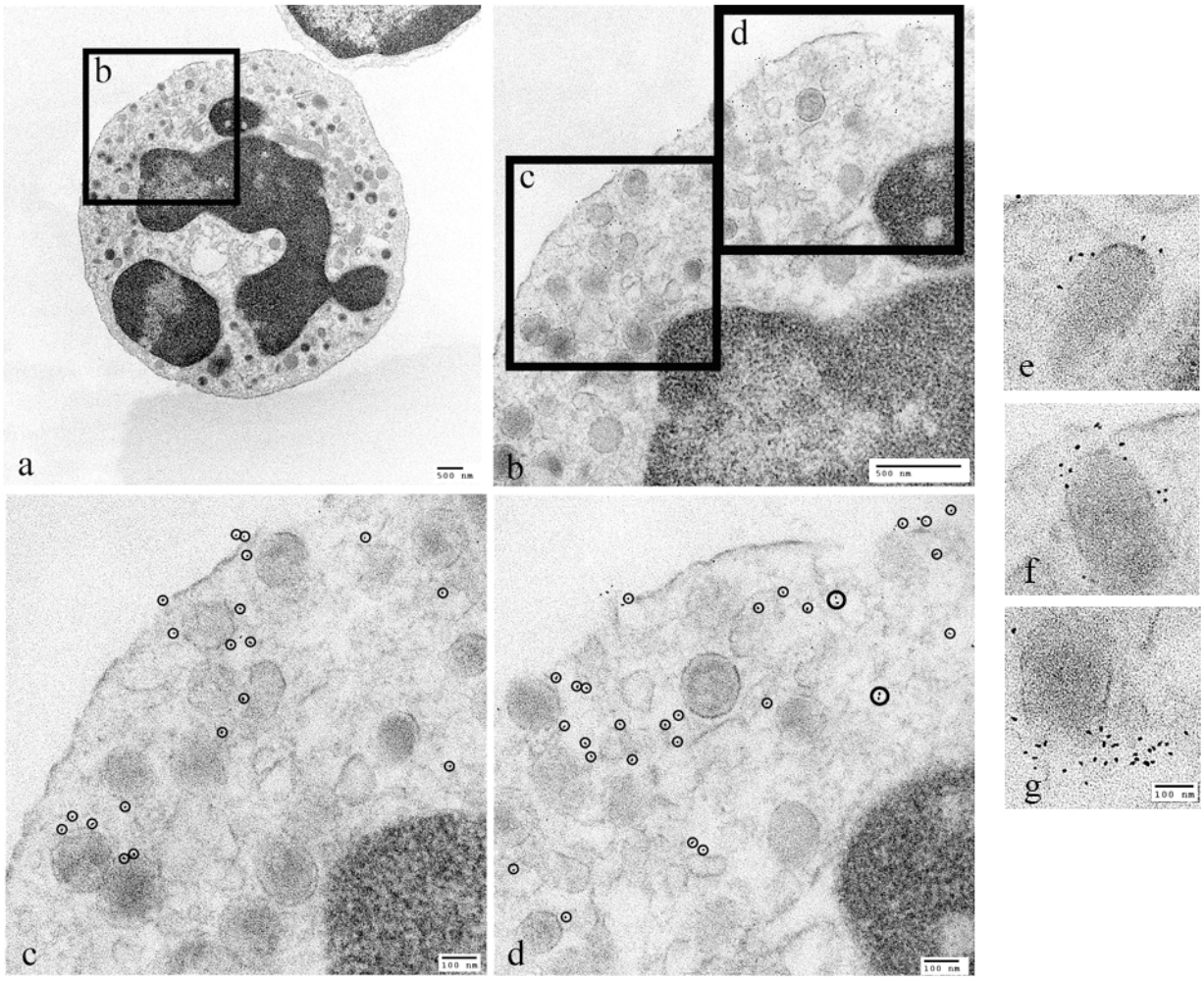

Fig.2. Immunolabeled neutrophil (a) and higher magnifications of a portion of the cell (b). Cores of Qdots are circled to illustrate localization in the cytoplasm (c,d). Qdots in proximity to cytoplasmic granules are shown in (e-g).

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