Human Platelet-Derived Mitogens. I. Identification of Insulinlike Growth Factors I and II by Purification and N° Amino Acid Sequence Analysis

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Human platelet lysates contained potent mitogenic activities for MCF-7 human breast-cancer cells in serum-free-defined media. Because these activities were not replaced by known platelet mitogens, such as platelet-derived growth factor or transforming growth factor β, we sought to identify the breast cancer cell mitogens by purification and N° amino-acid sequencing. Acetic acid extracts of outdated human platelets were concentrated by ammonium sulfate precipitation and fractionated on Sephadex G-50 and Bio-Gel P-10 columns in 0.5 mol/L acetic acid. Two major activities were resolved by molecular sieve methods and fractionated further by reverse-phase high-performance liquid chromatography (HPLC). Purifications (70,000 to 870,000-fold) were accomplished yielding mol wt 7,400 products that were homogeneous as determined by iodination, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and autoradiography. The factors were identified as insulinlike growth factor I (IGF-I) and II (IGF-II) and truncated IGF-I by N° amino acid microsequencing. In dose-response experiments, platelet-derived IGF-I and IGF-II promoted multiple divisions of the MCF-7 cells with ED_{50} values of 12 and 100 pg/mL, respectively. The specific activities and other bioassay characteristics of platelet-derived IGF-I and IGF-II were similar to those of recombinant-produced human growth factors. This is the first report of the purification of insulinlike growth factors from human platelet lysates.

PREVIOUSLY this laboratory demonstrated that lysates of outdated human platelets contained growth-factor activities for rat[1] and human[2] breast-cancer cells in culture. Microgram/milliliter concentrations of neutral pH extracts promoted continuous division of MTW9/PL rat and MCF-7 human cells under serum-free conditions. Lysates were shown also to be mitogenic for a wide variety of established epithelial and mesenchymal origin cell lines as well as containing growth factors for short-term cultures of human diploid fibroblasts.[2] Because of the diversity of cell types stimulated, we proposed that platelets contained many growth factor activities.[2]

Earlier reports had shown that platelets contained a potent mitogen[3-6] that was later purified to homogeneity by several methods[7-10] and that was established to be an A:B heterodimer with B-chain amino acid sequence homology to the expected protein product of the v-sis oncogene.[11,12] This factor, known as platelet-derived growth factor (PDGF), has since been identified in a homodimer form containing A chains.[13] Further heterogeneity of PDGF types was possible within the A chain structure.[14,15] Although mol wt 26,000 to 31,000 PDGF usually was considered a mitogen for mesenchymal cells,[16] our previous study[1] showed platelets contained a mammary epithelial cell activity of approximately mol wt 38,000, which might have been one of the forms of PDGF.

Platelets are also an important source of transforming growth factor β (TGFβ).[17] This dimeric mol wt 25,000 factor has been identified in both TGFβ1[17] and TGFβ2[18] forms and has been shown to influence mesenchymal cell growth positively and epithelial cells negatively.[19] Other studies confirmed that epidermal growth factor (EGF)/urogastrone[45] was present in the platelet structure, as was a mol wt 45,000 endothelial-cell growth factor.[20]

To determine which mitogens in platelets promoted growth of human breast-cancer cells, we used reverse phase high performance liquid chromatography (RP-HPLC) and a newly developed serum-free cell-culture bioassay method to purify the activities. The bioassay with MCF-7 and T47D human breast-cancer cells allowed a direct comparison of the potencies of five major functional families of nonlymphoid growth factors. The heterodimer form of PDGF, TGFβ1, and TGFβ2 were not mitogenic for these cell lines, whereas insulinlike growth factors I (IGF-I) and II (IGF-II), EGF, transforming growth-factor α (TGFα), and basic fibroblast growth factor (bFGF) promoted continuous cell divisions at picomolar to nanomolar concentrations.[21-23] Therefore platelet-derived breast-cancer cell growth factor(s) might have been related functionally or structurally to a homodimer form of PDGF or to any of the other mitogen groups that were active in this assay. Another possibility was a novel mitogen not previously characterized. To establish identity conclusively, we undertook the complete purification and identification by N° amino-acid sequencing. A preliminary report of these results has been presented.[24]

MATERIALS AND METHODS

Cell culture. Stock cultures of MCF-7 cells[25] (American Type Culture Collection, Rockville, MD) were maintained in a 1:1 (vol/vol) mixture of Ham's F12 and Dulbecco's Modified Eagle's media (DMEM, high-glucose formulation) supplemented with 2.2 g/L sodium bicarbonate (F12/DMEM), 15 mmol/L HEPES, pH 7.2, and 10% (vol/vol) fetal bovine serum (FBS) in a humidified atmosphere of 95% (vol/vol) air and 5% (vol/vol) CO_2 at 37°C. Cultures were passed every four to six days and seeded at 1.75×10^6/10-cm diameter plastic culture dish in 20 mL of medium. Stock cultures were assayed bimonthly for mycoplasma contamination using the MycoTect system purchased from GIBCO (Grand Island, NY); the tests were negative throughout this investigation.

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