

STRUCTURAL SIMILARITY OF PLATELET-DERIVED GROWTH FACTOR (PDGF) TO CANCER-TRIGGERING VIRUS-P²⁸SSIS: IMPLICATIONS IN ONCOTHERAPY

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ABSTRACT

The recent identification of a homology between the putative transforming protein of simian sarcoma virus (ssv) and platelet-derived growth factor (PDGF) is reviewed and discussed in the light of possible implications for radionuclidic tumour targeting as well as other possible onco-therapeutic modalities.

A STRIKING similarity between the putative transforming protein of simian sarcoma virus (ssv), p²⁸SSIS and the naturally occurring growth factor platelet-derived growth factor (PDGF) has been discovered. Antoniades¹ at the Blood Research Institute California, U. S. A. had reported the list of amino acids that constitute the PDGF sequence. Genetic theory permits one to postulate the DNA/RNA nucleotide sequences that correspond to any amino acid sequence in a polypeptide. Doolittle², a protein chemist at California University had created a computer data base NEWAT listing various known protein sequences. He fed the data of Antoniades¹ to his computer to determine whether there were any other proteins with known similar sequences. The computer identified a cancer-triggering virus (ssv) which was homologous with a protein with an amino acid sequence similar to PDGF.

Simultaneously another group working in the United Kingdom led by Waterfield³ reported the same discovery. This striking homology suggests that the virus has acquired cellular sequences which encode a growth factor identical or very similar to PDGF and that expression of this protein, mediates transformation by the virus.

PDGF is stored in the α granules of platelets which congregate at sites of damaged tissue for repair. The platelets lyse and release among other things, PDGF to initiate the growth of new cells to replace damaged ones. It is a potent mitogen for cultured fibroblasts, smooth muscle cells, and glial cells⁴. PDGF was isolated originally from whole human serum⁵ and subsequently from clinically outdated human platelets⁶⁻⁸ and from human platelet-rich plasma⁹. It is a heat-stable (100°C), cationic (isoelectric point, pI, 9, 8) polypeptide with a range of molecular weights for non-reduced active form from 28,000 to 35,000 as judged by

analytical sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). It binds to specific saturable cell-surface receptors and stimulates tyrosine-specific kinase activity^{10, 11}. Other early cellular responses include changes in ion fluxes, cyclic AMP accumulation mediated by synthesis of E-type prostaglandins, altered phosphorylation of intracellular proteins, stimulation of actin reorganization and membrane ruffling, modulation of epidermal growth factor binding and expression of specific genes.

Biologic activity during purification was assessed by the ability of PDGF to stimulate DNA synthesis in cultures of BALB/c-3T3 (clone A30) cells⁶. The specific activity of purified PDGF is estimated at about 3000 units per microgram of protein, and that of platelet lysate is about 0.03 unit per microgram of protein. One unit of PDGF activity is defined as the amount required to induce 50% of the cells to synthesize DNA.

Two major bands of PDGF activity, corresponding to protein bands of 35,000 (PDGF-1) and 32,000 (PDGF-2) daltons, were recovered from the final stage of purification (SDS-PAGE) which matches at the following positions to p²⁸SSIS protein sequences².

(1) PDGF-1 matches p²⁸SSIS at 18 of 29 positions identified by protein sequencing.

(2) PDGF-2 matches p²⁸SSIS at 26 of 31 positions at the PDGF-2 amino terminus and at 35 of 39 positions at the amino terminus of a 14,000-dalton PDGF-2 cyanogen bromide fragment (total match at 61 to 70 identified positions).

Reduced forms of PDGF-1 and PDGF-2 have a molecular weight 18,000 and active PDGF may either be a single protein formed by disulphide linkage of these two peptides or consist of two proteins each of which is a disulphide linked dimer of the homologous peptide fragments.

The ssv gene product and human PDGF have been

demonstrated by Robbins *et al.*¹² to have striking structural and immunological similarities. The primary translation product p^{28^{sis}} of 28,000 MW is rapidly cleaved to polypeptides of 11,000 and 20,000 MW, the later being related to the C terminus.

The 20,000 MW polypeptide derived from p^{28^{sis}} is very similar to PDGF-2, sequence correspondence beginning at position 67 for about 160 aminoacids. Immunological studies have further confirmed the conformational similarity of the two molecules using anti-PDGF serum or using a serum directed against a synthetic peptide representing¹³ residues 139 to 155 of p^{28^{sis}}.

Both p^{28^{sis}} as well as PDGF share dimeric structure and susceptibility to proteolysis. Functionally both exert pleiotrophic effects on cellular metabolism and can induce sustained cell replication. 'sis' oncogene activation thus has been implicated as a step on oncogenesis.

Doolittle² concludes that the v-sis transforming gene in the monkey is the result of the SSV recombination with the host cell gene or genes encoding PDGF itself or a closely similar protein.

Viral invasion of host cells with subsequent malignant transformation is now conceptualized to introduce DNA fragments which get incorporated in the host cell's genetic machinery, commanding it to produce a protein sequence virtually identical to naturally occurring PDGF. Excessive PDGF production leads to unrestricted growth of cells with PDGF sensitive receptors located in the vicinity. In addition, it is possible that intracellular PDGF may even bypass surface PDGF receptors. Unrestricted growth under the effect of PDGF may lead to the formation of a tumour. Although this mechanism was first identified in the monkey sarcoma as described above, a mitogen structurally, functionally and immunologically related to PDGF has also been partially purified from human osteosarcoma cells (U-2 OS)^{3,14}. In addition, the growth factor released by simian virus 40 (SV-40)-transformed BHK cells¹⁵ also has PDGF-like properties¹⁶. Search is now being mounted for a similar mechanism in other tumours linking cancer genes, mitogenic growth factors and uncontrolled cell growth and multiplication.

If such a mechanism proves to be widespread this could lead to a greatly advanced understanding of how cancers develop and may also have implications in imaging and treatment.

There has been recent interest in the possibility of imaging tumours using radio labelled antibodies directed against tumour specific antigens and this has possible implications for therapy if the antibody

'missile' carries a lethal 'warhead'. After the early attempts of Pressman¹⁷ and Mach¹⁸ using polyclonal antisera, several studies using monoclonal antibodies have been reported from Europe and United States using antibodies directed against cell surface antigen. Since surface antigens may be shed into the blood stream mopping up the antibodies before they reach the target cells, and also because the fundamental change in cancer occurs in the nucleus, the cell surface alteration being secondary, we had suggested that malignant cell nuclear antigens may prove to be more profitable targets rather than cell surface antigens¹⁹.

This suggestion has been strengthened by the recent recognition that expressed cell surface antigens may be multiple and different because of antigenic modulation^{20,21}. It now appears to us that the cancer genes which are closely linked to known growth promoting mitogenic factors such as PDGF should be intensively investigated as possible targets and their distribution in various human tumours should be studied. Antibodies can also be deployed against the mitogenic factors or their receptors, and this may be more feasible than using antibodies against the oncogene, because it appears that oncogenes may also exist in normal cells but are not 'switched on'²². Endogenous growth factors in naturally occurring areas of tissue proliferation may compete with the oncogene induced mitogenic factors when antibodies are administered but the relative concentration and epitopic configuration of these targets needs to be analysed in animal models.

Apart from this therapeutic implication, there are other possible implications of the recent recognition of the PDGF mechanism.

It may be possible to pharmacologically block the action of PDGF either at the receptor site of tissue cell surfaces or better still intracellularly. Apart from competitive block it may be possible to destroy PDGF *e.g.* by cleaving the S-S bond using radiation or chemicals but whether this will really be feasible needs to be experimentally tested.

The search for the function of other oncogenes may well unravel further interactions with the mitogenic cascades induced by growth factors thus suggesting more possible treatment modalities in cancer.

Doolittle *et al.*² raised certain fundamental questions in relation to their discovery such as whether the PDGF activity is present only in the dimeric configuration or even in the single chain, and whether the viral oncogene related protein acts through the PDGF cell membrane receptor?

They have also referred to other known linkages

between oncogenes and cell growth. Shih²³ reported an oncogene with associated GTP-binding (guanosine triphosphate) activity; the SRC gene product is homologous to cyclic AMP kinase²⁴ and certain oncogenes code for protein kinases which can phosphorylate tyrosine residues. Transferrin-receptors have been often identified in association with tumours (Greaves, personal communication, 1981) and Goubin²⁵ have described a possible homology between transferrin and the Blym gene product.

The recognition of these oncogenes and their manifold relationship to cell growth is thus beginning to be unravelled. It appears reasonable to predict that the information now being discovered will eventually transform oncotherapy.

18 July 1984; Revised 17 September 1984

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NEWS

'TEST-TUBE' SKIN

. . . "A medical team has helped save two severely burned young brothers by taking tiny patches of skin from their bodies, growing the patches into large sheets and grafting them back over the burns. Researchers say the new technique in which such skin patches are induced to grow first in test tubes and later on large strips of gauze in a laboratory, could represent a major advance in the treatment of extensive burns . . . Doctors at Massachusetts General Hosp.

who developed the treatment said . . . that they had used it to replace more than half the skin area of each of the young brothers, who were burned over 97% of their bodies." [(Lawrence K. Altman in *New York Times*, 16 Aug 84, p. A1, A18) (Reproduced with permission from Press Digest, *Current Contents*®, No. 50, December 10 1984. Published by the Institute for Scientific Information®, Philadelphia, PA, USA)]