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Secretory factors of human neuroblastoma (IMR-32) and human glioblastoma (U87MG) cell lines induce neurite outgrowths in PC12 cells

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Neurite outgrowth is essential for the communication of the nervous system. The rat Pheochromocytoma (PC12) cells are commonly used in the neuronal cell study. It is well known that exogenous stimuli such as Nerve Growth Factor (NGF) induce neurite outgrowth. In the present study it has been investigated whether or not the conditioned medium from human neuroblastoma cell line (IMR-32) and human glioblastoma cell line (U87MG) may augment neurite outgrowth in PC12 cells. PC12 were cultured with and without conditioned media of IMR-32 and U87MG. The result showed that both the conditioned media induce neurite outgrowth within 48 hr and stops further proliferation of PC12 cells. However no outgrowth was noted in PC12 cells incubated without conditioned medium. In conclusion, it is shown that both the conditioned media (IMR-32 and U87MG) have the potential to induce the neurite outgrowth in the PC12 cells.

Keywords: Conditioned medium, Neurites, Pheochromocytoma

Pheochromocytoma (PC12) cells are commonly used in the study of neuronal cells[1]. It is one of the most widely used cell lines for cellular signaling system[2-4]. The striking feature of the PC12 cell line is its ability to differentiate into neuronal cells in response to Nerve Growth Factor (NGF)[5-7], Glial Derived Neurotrophic Factor (GDNF)[8], and cAMP[9]. The mechanism of neurite outgrowth induced by NGF has been investigated[2,5]. Culture medium conditioned over C6 glioma cells contain factors, which induce neurite outgrowth in PC12 cells[10-12]. Neuronal differentiation of PC12 cells inducing molecule or combination of molecules present in a medium conditioned by culture of rat sciatic nerves has been well documented[13]. Based on these observations in the present study, it has been investigated whether or not a conditioned medium from IMR-32 and U87MG cell line may augment neurite outgrowth in PC12 cells.

Materials and Methods

Cell culture— PC12 cells (rat Pheochromocytoma) were routinely maintained in DMEM (Gibco BRL) medium supplemented with 20% new born calf serum, (Sigma MO. USA), 10% heat inactivated horse serum (Sigma MO. USA), 10µl/ml penicillin, 25µg/ml streptomycin and 25µg/ml amphotericin B (Himedia, India) (complete culture medium). Cells were routinely sub-cultured every 4-5 days.

Cell lines— IMR-32 (human neuroblastoma) and U87MG (Human glioblastoma) cell lines were purchased from the National Center for Cell Science (NCCS), Pune, India. Both cell lines were cultured and maintained in DMEM medium supplemented with 10% new born calf serum, 10µl/ml penicillin, 25µg/ml streptomycin and 25µg/ml amphotericin B.

Preparation of conditioned medium— IMR-32 and U87MG (3×10^{6}) cells were seeded in T-25 tissue culture flask (Orange Scientific, USA) in 10 ml DMEM medium and were allowed to grow for 3 to 4 days or till confluence was observed. When 90 % confluence was obtained, the medium was removed and centrifuged at 2000 rpm for 20 min to remove the suspended cells. The supernatant collected was filtered through 0.22 µm membrane syringe filter (Laxbro, India), and the conditioned medium was stored at 4°C till further use.

Treatment of PC12 cells with conditioned medium— PC12 (2×10^{4}) cells were seeded per ml of DMEM medium in 24 well tissue culture plate. Prior

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to experimental treatment, the cells were grown overnight in complete culture medium. This provides sufficient time for cells to attach to substratum. On the next day this medium was replaced with 1 ml of the conditioned medium of IMR-32 and U87MG cell lines (added separately). These cells were then kept in 5% CO_2 + 95 % air at 37°C in humidified environment for differentiation and were microscopically observed.

Results

The induction of neurite outgrowth in PC12 was examined over 25-days (0-25 days) without addition of conditioned medium or any other inducer. No neurite outgrowth was observed during this period (Fig.1). Exposure of PC12 cells to the conditioned medium of IMR-32 induced PC12 differentiation into neuronal cells, within 48 hr (Fig. 2). PC12 cells underwent morphological changes similar to those induced by NGF, which is a well-known inducer of neuronal differentiation in these cells[6,14]. Similarly conditioned medium of U87MG induced neurite outgrowth in PC12 cells (Fig. 3). Both the conditioned media induced permanent changes in PC12 cells, which lost viability and neurite outgrowths underwent degeneration if conditioned medium was removed intermittently.

Discussion

The results of the present study will be helpful for the identification of molecules present in the conditioned medium of IMR-32 and U87MG. These secretory factors may have the properties as that of other neurotrophic factors. The astroglial cells cultured from mouse brain synthesize and secrete NGF[15]. Other NGF like molecules [e.g. Brain-Derived Neurotrophic Factor (BDNF) and Neurotrophin-3 (NT-3)] may be secreted by these cells and that some measurable biological responses arise from these molecules. However PC12 cells have not been reported to express Trk receptors (Trk B and Trk C) [6] for other members of the neurotrophic family. Thus, the secretory factors from IMR-32 and U87MG must be mimicking the NGF to occupy its receptor and switch on the differentiation cascade of events.

Purines nucleosides, such as adenosine, have increasingly been recognized as important intercellular trophic signaling molecules in the nervous system. It has already been reported that the PC12 cells possess A_2A receptor for adenosine[16]. Adenosine, inosine and guanosine have all been reported to induce neurite outgrowth or to enhance NGF induced neurite outgrowth synergistically[17]. Higher intracellular concentration of adenosine induces neurite outgrowth. The conditioned medium may have the higher concentration of purines nucleosides or cyclic nucleotides, which induces the neurite outgrowth in PC12 cells, or their concentration may be lower but they potentiate the activity of other factors present in the conditioned medium.

The secretory factors in the conditioned medium of IMR-32 and U87MG may prove useful for the studies on neuroprotection and for the treatment of various disorders. GDNF used for the treatment of Parkinson's disease, has shown significant improvement in several of cardinal symptoms of Parkinson's disease[18].

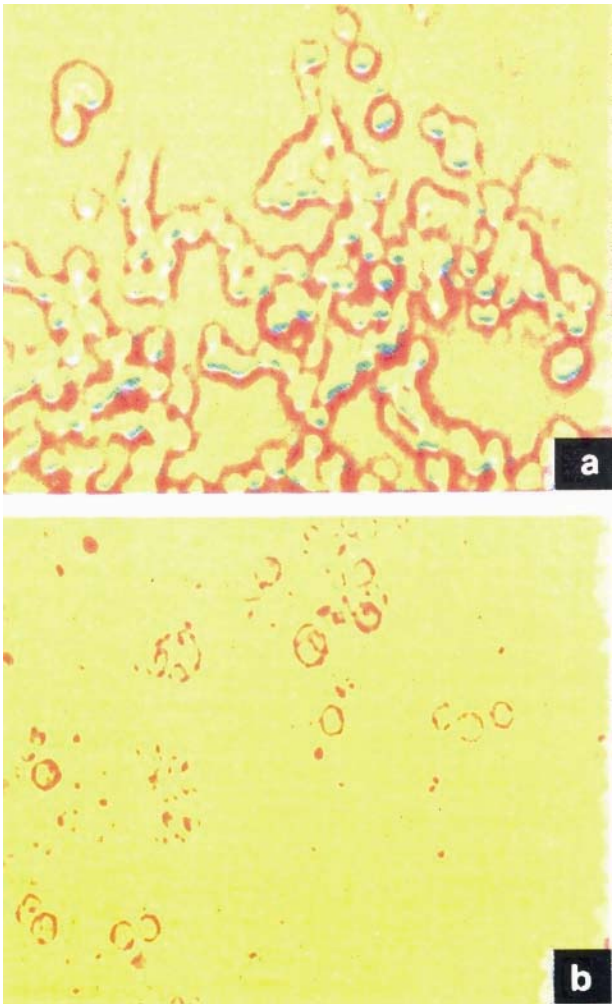

Fig. 1— PC12 cells do not show any neurite outgrowth at the time of culturing (a) till 25 days (b) without the addition of any stimulator.

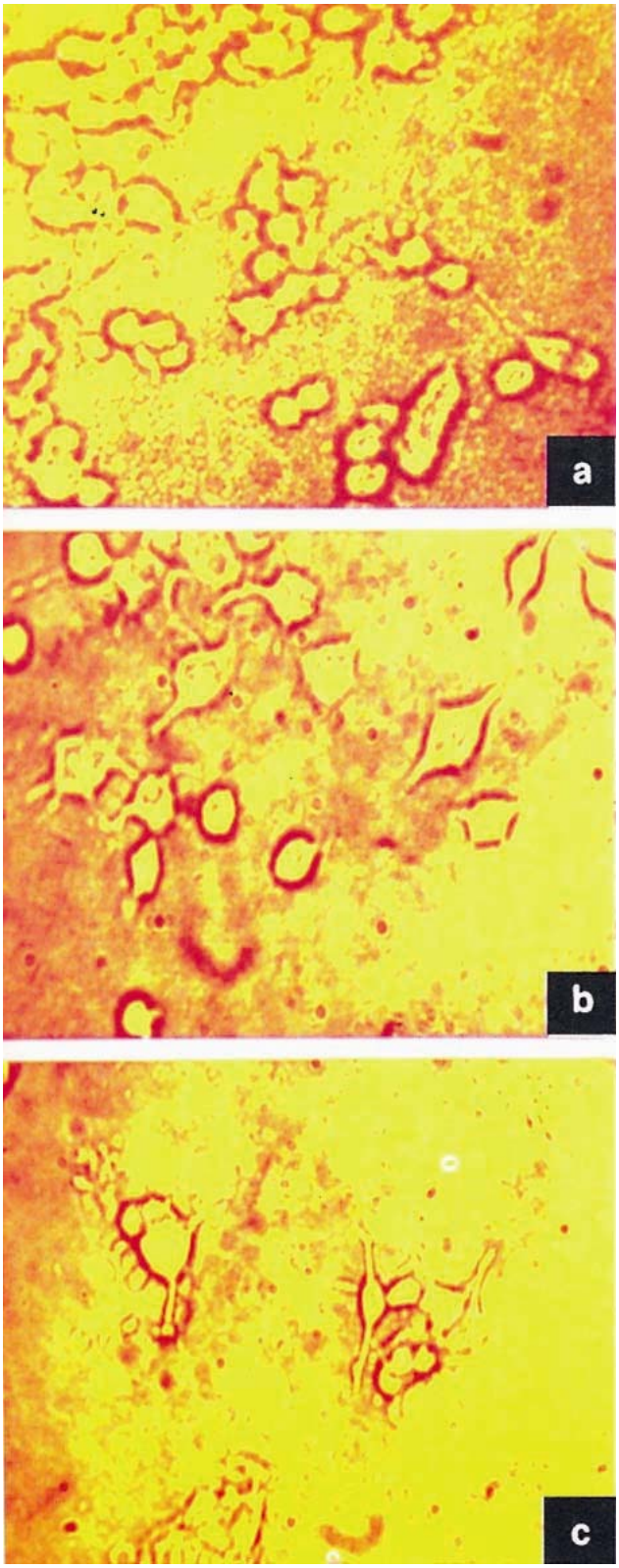

Fig. 2— PC12 cells at the time of culture (a) starts neurite outgrowths at 24 hr (b) and further extends at 48 hr (c) when treated with conditioned medium of IMR-32.

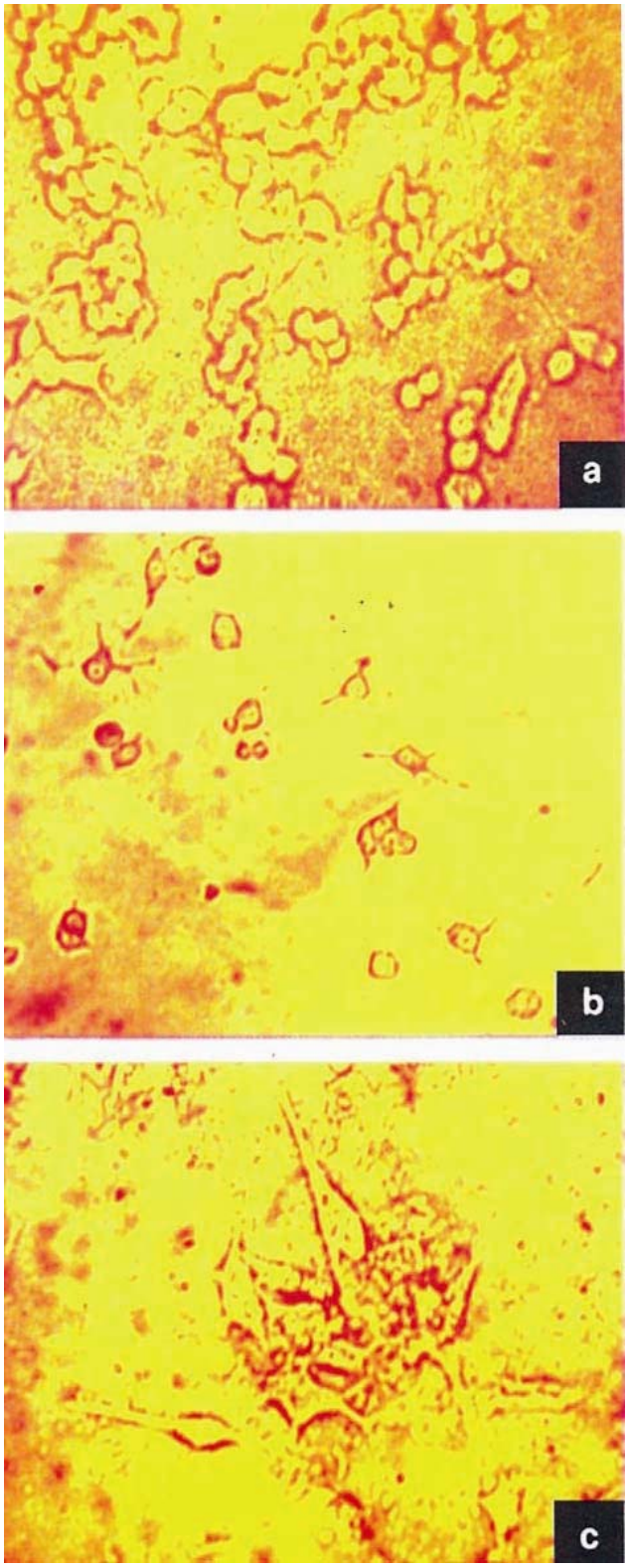

Fig. 3— PC12 cells at the time of culture (a) starts neurite outgrowths at 24 hr (b) and further extends at 48 hr (c) when treated with conditioned medium of U87MG.

Differentiation of PC12 cells after addition of the conditioned medium IMR-32 and U87MG, indicates that these secretory factors can play a very important role in various biochemical pathways leading to differentiation. The identification and characterization of these secretory factors and the exploration of the biochemical pathways they follows, are under investigation.

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