IGF-I stimulates chemotaxis of human neuroblasts. Involvement of type 1 IGF receptor, IGF binding proteins, phosphatidylinositol-3 kinase pathway and plasmin system

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Abstract

SH-SY5Y human neuroblastoma cells express IGF receptors, IGFs and IGF binding proteins (IGFBPs), and provide a model for studying the role of the IGF system in human neuronal development. We investigated the effect of IGF-I and des(1-3)IGF-I on the motility of SH-SY5Y cells by a cell migration assay based on the assessment of the number of cells which migrated across 8 μ m pore size membranes and around an agarose drop. IGF-I and des(1-3)IGF-I stimulated neuroblast chemotaxis in a dose-dependent manner. Treatment of cells with these agents for 24 h resulted in a significant increase (IGF-I by 70% and des(1-3)IGF-I by 90%; $P < 0.0001$) in cell motility relative to control conditions. Addition of monoclonal antibody against type 1 IGF receptor (α -IR3), significantly ($P < 0.05$) reduced the cell motility induced by IGF-I (by 30%) and des(1-3)IGF-I (by 70%). Wortmannin, a specific inhibitor of phosphatidylinositol (PI)-3 kinase

intracellular signalling, also reduced the IGF-stimulated cell migration (by over 40%, $P < 0.01$), indicating a key role of the PI-3 kinase pathway in mediating the IGF effect on neuroblast migration. Finally, cell treatment with plasminogen (PLG) markedly enhanced neuroblast migration (by over 200%, $P < 0.01$), whereas incubation with the PLG inhibitor 4-(2-aminoethyl)-benzenesulphonyl fluoride reduced cell motility (by 80%, $P < 0.01$), thus suggesting an involvement of PLG-dependent IGFBP proteolysis in the regulation of neuroblast motility. In conclusion, IGF-I is a potent stimulator of neuroblast migration through the activation of type 1 IGF receptor and the PI-3 kinase intracellular pathway. IGFBPs and the plasmin system seem to play a role in cell motility, although the nature and the extent of their involvement has yet to be elucidated.

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Introduction

The insulin-like growth factors (IGF)-1 and -II play a key role in cell cycle progression, cell proliferation and tumour progression (Humbel 1990, LeRoith *et al.* 1995a, Resnicoff *et al.* 1995). Most of the effects of IGFs are mediated by binding to type 1 IGF receptor, whereas type 2 IGF receptor is mainly involved in the clearance of IGF-II (LeRoith *et al.* 1995b, Stewart & Rotwein 1996). In all biological fluids, IGFs are bound to at least six different high affinity binding proteins (termed IGFBP-1 to -6) which prolong IGF half-life, counteract the insulin-like hypoglycaemic effect of IGFs, maintain a reservoir of IGFs in the circulation, transport IGFs from the circulation to peripheral tissues, modulate IGF action, and also exert IGF-independent actions (Cianfarani & Holly 1989, Jones & Clemmons 1995). The complexity of the IGF system is further increased by the intervention of specific proteases which, by fragmenting IGFBPs, reduce their affinity for

IGFs and eventually lead to augmented IGF bioavailability (Hossenlopp *et al.* 1990, Giudice 1995).

There is increasing evidence that IGFs are also able to stimulate cell motility. Many types of cells, such as endothelial cells, keratinocytes, osteoblasts, rhabdomyosarcoma cells, epithelial cells, trophoblasts, melanoma cells, breast cancer cells, smooth muscle cells, and carcinoma cells, migrate towards a source of IGFs or display increased motility in the presence of these factors (Leventhal & Feldman 1997). Type 1 IGF receptor has been implicated as a mediator of IGF-stimulated cell motility (Stracke *et al.* 1989) and signalling mechanisms involving type 1 receptor autophosphorylation, tyrosine phosphorylation of insulin receptor substrate family (IRS-1 to IRS-4), activation of phosphatidylinositol (PI)-3 kinase, integrin-dependent adhesion, and tyrosine phosphorylation of the focal adhesion proteins, such as paxillin and focal adhesion kinase (FAK), are involved in IGF-stimulated cell migration (Leventhal & Feldman 1997).

Neuroblastoma is the second most common solid tumour in childhood and accounts for 10% of all juvenile cancer (Brodeur & Castleberry 1993). This malignant embryonic neoplasm is of neural crest origin and primarily consists of two cell types: N cells, or neuroblasts, and S cells, representing Schwann cells, epithelial cells, and melanocytes. The IGF system plays a major role in the biology of neuroblastoma (Cianfarani & Rossi 1997). The IGFs are, in fact, intimately involved in growth (El-Badry *et al.* 1989, 1991) and differentiation (Recio-Pinto & Ishii 1988, Pahlman *et al.* 1991) of neuroblasts acting in an autocrine (Martin & Feldman 1993) and paracrine (Leventhal *et al.* 1995) fashion. In addition, we and other authors have recently demonstrated that human neuroblastoma cells are able to produce IGFBPs (Bernardini *et al.* 1994, Babajko & Binoux 1996), particularly IGFBP-2, -4 and -6, which modulate both the growth- and differentiation-promoting effects of IGFs (Cianfarani *et al.* 1996, Babajko *et al.* 1997). Finally, neuroblastoma cells secrete tissue-type plasminogen activator which by transformation of plasminogen (PLG), is able to generate plasmin that acts as an IGFBP protease (Neuman *et al.* 1989, Lalou *et al.* 1994, Angeloz-Nicoud & Binoux 1995). The addition of PLG in neuroblast cultures has recently been shown to induce IGFBP-2 proteolysis and, by reducing the IGFBP-2 affinity for IGF-II, to stimulate neuroblastoma cell proliferation (Menouny *et al.* 1997). Plasminogen activator is also involved in motility of neural crest cells, which produce high levels of this serine protease during their migration from the neural tube to the ventral areas of head and neck where cytodifferentiation occurs (Valinsky *et al.* 1985).

The SH-SY5Y human neuroblastoma cell line is an N cell line thought to have arisen from the neural crest (Biedler *et al.* 1978). SH-SY5Y cells express insulin, type 1 and type 2 IGF receptors, undergo morphological and functional neuronal differentiation when exposed to appropriate stimuli and, therefore, are often used as a model for studying neuronal development (Pahlman *et al.* 1990) and the interactions between the IGF system and neuroblasts (Pahlman *et al.* 1991, Martin & Feldman 1993, Sumantram & Feldman 1993, Cianfarani *et al.* 1996). In SH-SY5Y cells, IGFs have been shown to exert an anti-apoptotic action (Matthews & Feldman 1996), and to induce essential steps preliminary to cell migration, such as actin polymerization and membrane ruffling with protrusion of the cell leading edge (Lauffenburger & Horwitz 1996, Leventhal *et al.* 1997). This IGF action on cell architecture is inhibited by the addition of wortmannin, a fungal toxin that selectively blocks PI-3 kinase activity (Kotani *et al.* 1994).

In the present study we have investigated the effect of IGF-I on the motility of SH-SY5Y human neuroblastoma cells, and the role of type 1 IGF receptor, IGFBPs, plasminogen, and PI-3 kinase in the IGF-stimulated neuroblast migration.

Materials and Methods

Cell culture

Human neuroblastoma SH-SY5Y cells were cultured in minimum essential medium (MEM) (Gibco, Grand Island, NY, USA) supplemented with 10% FCS (Gibco) in the presence of 2 mM glutamine, 100 IU/ml penicillin, and 50 µg/ml streptomycin. Cultures were maintained in a humidified incubator at 37 °C with 5% CO₂ atmosphere. At the end of the exponential growth phase (72 h doubling time), cells were dispersed using 0.02% EDTA (Sigma, St Louis, MO, USA). In each assay, FCS-free RPMI 1640 with glutamine was used.

IGF-I and des(1-3)-IGF-I were from GroPrep (Adelaide, Australia), the anti type I IGF receptor monoclonal antibody, α -IR3, was purchased from Calbiochem (San Diego, CA, USA). To inhibit IGF binding to insulin receptor, we used the monoclonal antibody, MA-10, kindly supplied by Dr G Sesti, Department of Internal Medicine, 'Tor Vergata' University, Rome (Forsayeth *et al.* 1987). The antiproliferative factor, cytosine arabinoside (Ara-C), was purchased from Pharmacia-Upjohn (Bridgewater, NY, USA). The optimal Ara-C concentration for inhibiting cell proliferation was determined by sulphorhodamine B assay, and preliminary experiments revealed that Ara-C maintained its inhibitory effect on DNA synthesis for 48 h and had no effect on cell motility. Tissue culture flasks and multiwell plates were purchased from Falcon (Lincoln Park, NJ, USA). For migrating assay cell culture, polyethylene terephthalate (PET) inserts with 8.0 µm pore size and 1.0×10^5 pores/sq.cm (Falcon) were used; the inserts were put into 6-well plates. Wortmannin, PLG and 4-(2-aminoethyl)-benzenesulphonyl fluoride (plasminogen inhibitor, Pc) were obtained from Sigma.

Migration assay with cell culture inserts

Migration assay was a modification of Harvath's method (Harvath *et al.* 1980). Cells (at or near confluency) were dispersed with 0.02% EDTA and replated in culture inserts in serum-free medium. The final cell density was assessed by a haemocytometer, counting the viable trypan-blue excluding cells. The lower wells of the 6-well plates were loaded with 3 ml RPMI 1640 containing Ara-C (625 ng/ml). The cell culture inserts were placed over the 6-well plates and loaded with 1.5 ml RPMI 1640 plus Ara-C (625 ng/ml) and containing 4.5×10^5 cells. After 24-h incubation at 37 °C to allow the cell attachment to the insert membranes, IGF-I (1 to 100 nM) and des(1-3)IGF-I (1 to 100 nM) were added in the lower wells. In some assays, cells were preincubated with α -IR3 (1 µg/ml), MA-10 (6.6×10^{-7} M) or wortmannin (50 nM) for 60 min at 37 °C. PLG (5 µg/ml) and Pc (0.075 mM) were added 8 h after the addition of IGFs.

Figure 1 Migration of SH-SY5Y cells: the effect of IGF-I and des(1–3)IGF-I. Each migration stimulatory effect is compared with the response of cells to des(1–3)IGF-I (5 nM) in the same assay, which, eliciting the maximal response, is assigned an arbitrary value of 100%. * $P < 0.001$ vs control. CNTRL, control.

To determine the direction of migration, cells were plated in the upper surface of the insert membranes and the IGFs were added in the lower well only, in the upper well only, or in both wells. After 24-h incubation, cells were fixed with 10% trichloroacetic acid (Sigma), and cells on the upper surface of the inserts were removed by a sterile cell scraper (1.8-cm blade, Falcon), in order to leave the cells which had migrated across the membrane and which were present on the lower surface. The cells were then stained with 6% GIEMSA (Merck KGA, Darmstadt, Germany), and membranes were removed and put on a glass slide. The number of migrated cells with whole body outside the pores was assessed in a blinded fashion by three observers who counted, with an inverted microscope fitted with a grid eyepiece at a total magnification of $\times 20$, twenty fields for each slide. All experiments were performed in triplicate and were repeated a minimum of three times.

Migration assay with agarose drop

Cell migration was visualized by measuring the extent of neuroblast migration from an agarose drop using a modified method described by Varani *et al.* (1978). Briefly SH-SY5Y cells were resuspended at $30 \times 10^6/\text{ml}$ in RPMI 1640 media containing 10% FCS and 0.3% low melting point agarose maintained at 37°C to prevent setting of the agarose; 2 μl of the cell suspension were applied to the centre of the wells within a 24-well tissue culture dish (Falcon), which was then placed at 4°C for 30 min to allow the agarose to solidify. Thereafter, agarose drop was covered with 0.4 ml serum-free media and maintained for 24 h at 37°C . Eventually, IGFs were

added and the plate maintained at 37°C for 24 h. Photomicrographs of the agarose drop assay were taken after 24-h incubation.

Statistics

Differences of means of at least three different experiments performed in triplicate and read by three independent observers were assessed using one-way analysis of variance (ANOVA). Significance was assigned for $P < 0.05$. A computer program was used for all statistical calculations (BMPD, statistical software, SOLO 3.0, Los Angeles, CA, USA).

Results

IGFs stimulate migration of SH-SY5Y neuroblasts

IGF-I significantly stimulated neuroblast migration in a dose-dependent manner, increasing cell motility by over 70% ($P < 0.0001$). Des(1–3)IGF-I, an IGF-I analogue five to ten times more potent than IGF-I in stimulating DNA and protein synthesis because of its lower binding affinity for IGF-BPs, was significantly more potent than IGF-I in enhancing cell motility (Fig. 1). The migration promoting effect of des(1–3)IGF-I was observed at concentrations as low as 1 nM and reached a peak at a concentration of 5 nM (more than 90% increment), with a reduction at higher concentrations, thus suggesting a chemotactic action. At low concentrations of a chemoattractant, no cell movement occurs. At higher concentrations, the cells are able to distinguish a difference in the concentration gradient of substance, and thus move in a directed fashion. At still

higher concentrations, the gradient is no longer distinguishable and the cell does not move towards the chemo-attractant. The potent effect of des(1–3)-IGF-I on cell migration suggests that IGFBPs may inhibit IGF-stimulated cell motility. The IGF stimulatory action on cell motility was independent of the mitogenic action, since cell proliferation was blocked by the addition of the DNA polymerase inhibitor, Ara-C. The IGF stimulatory effect on neuroblast motility was confirmed by the agarose drop assay (Fig. 2).

Type 1 IGF receptor mediates the IGF-induced cell migration

The addition of the monoclonal antibody, α -IR3, which specifically inhibits IGF binding to type 1 IGF receptor (IGF-IR), induced a significant reduction of both IGF-I- (by approximately 30%, $P < 0.05$) and des(1–3)IGF-I-induced cell migration (by approximately 70%, $P < 0.05$), thus suggesting that most of the IGF effect on neuroblast motility is mediated by the activation of IGF-IR (Fig. 3). We also tested the effect of the inhibition of insulin receptor on neuroblast migration using the monoclonal antibody, MA-10, which failed to inhibit cell motility.

The IGF-stimulated migration is directional

To determine whether the IGF-induced cell motility is directional towards a concentration gradient of IGFs (chemotaxis) or is a random movement (chemokinesis), we performed a migration assay in which IGF-I (10 nM) was added to the lower wells only, the upper wells only, both sets of wells, or neither. As shown in Fig. 4, the addition of IGF-I to the upper well or to both wells led to significantly less migration compared with that observed when IGF-I was present in the lower well only. These experiments demonstrated that IGF-I stimulated a primarily chemotactic response.

IGF-stimulated neuroblast migration is mediated by PI-3 kinase activation

To test whether PI-3 kinase is involved in neuroblast migration, a migration assay using its specific inhibitor, wortmannin, was performed. Wortmannin was able to reduce significantly the IGF-I- (by approximately 40%, $P < 0.01$) and des(1–3)IGF-I-stimulated neuroblast migration (by approximately 50%, $P < 0.01$) (Fig. 5).

Plasminogen system plays an active role in IGF-induced neuroblast migration

IGFBP-2 is the major IGFBP secreted by human neuroblasts and undergoes proteolysis by PLG. To determine the role of IGFBP proteolysis in IGF-stimulated neuroblast motility, cells were treated with PLG (5 μ g/ml) in the presence or not of its specific inhibitor pefablock

Figure 2 Agarose drop assay: the IGF stimulatory effect on neuroblast motility. SH-SY5Y cells in an agarose drop after different treatment conditions: (A) serum-free medium alone; (B) serum-free medium plus 10 nM IGF-I, and (C) serum-free medium plus 100 nM IGF-I.

(Pc)-SC (4-(2-aminoethyl)-benzenesulphonyl-fluoride) (0.075 mM), a serine protease inhibitor previously shown to be innocuous to neuroblasts at this concentration (Menouny *et al.* 1997). PLG significantly enhanced both the IGF-I- (by approximately 250%, $P < 0.01$) and des(1–3)IGF-I-induced (by approximately 200%, $P < 0.01$) cell

Figure 3 Inhibition of SH-SY5Y cells migration induced by the anti type 1 IGF receptor antibody, α -IR3 (1 μ g/ml). Cell migration in response to IGF-I (10 nM) and des(1–3)IGF-I (5 nM) was determined in the presence and the absence of the type 1 IGF receptor blocking antibody, α -IR3. Taking the total number of migrated cells in response to des(1–3)IGF-I (5 nM) with no added antibody as 100% migration (maximal stimulatory effect), α -IR3 significantly reduced the response to both IGF-I and des(1–3)IGF-I; * $P < 0.05$. CNTRL, control.

migration, and this effect was reduced by preincubation with Pc, which significantly inhibited cell motility (by approximately 80%, $P < 0.01$) in respect to either IGF-I

or des(1–3)IGF-I, thus suggesting an involvement of PLG-dependent IGFBP proteolysis in modulating the IGF-dependent neuroblast migration (Fig. 6).

Figure 4 Directional migration of SH-SY5Y cells. The graph depicts the effects on SH-SY5Y cell migration of adding IGF-I (10 nM) to the lower wells, the upper wells, neither well, or both wells, and was performed as described in Materials and Methods. The number of migrated cells when IGF-I was present in the lower wells only is assigned a relative value of 100% and is a measure of chemotaxis. Migration in response to the addition of IGF-I to only the upper wells or to both wells is a measure of chemokinesis, stimulation of migration in the absence of a concentration gradient. IGF-I is more potent in stimulating migration when there is a positive concentration gradient towards which the cells migrate; * $P < 0.01$.

Figure 5 Inhibition of SH-SY5Y cells migration by wortmannin. Cell migration in response to IGF-I (10 nM) and des(1–3)IGF-I (5 nM) was determined in the presence and in the absence of wortmannin (Wort.) (50 nM), a specific inhibitor of PI-3 kinase. Taking the total number of migrated cells in response to des(1–3)IGF-I (5 nM) with no added wortmannin as 100% migration (maximal stimulatory effect), wortmannin significantly reduced the cell response to both IGF-I and des(1–3)IGF-I; * $P<0.01$. CTRL, control.

Discussion

We have previously reported an IGF-I growth and differentiation promoting effect in SH-SY5Y cells (Cianfarani

et al. 1996); in this study we provide direct evidence that IGF-I also stimulates motility of these human neuroblasts, exerting a chemoattractant activity. This finding is consistent with the observation that IGF-I induces actin

Figure 6 Effect of plasminogen on SH-SY5Y cells migration. Cell migration in response to IGF-I (10 nM) and des(1–3)IGF-I (5 nM) was determined in the presence of plasminogen (PLG, 5 µg/ml) in the presence or in the absence of the plasminogen inhibitor, Pc (0.075 mM). Taking the total number of migrated cells in response to des(1–3)IGF-I (5 nM) with no added PLG as 100% migration (maximal stimulatory effect), PLG significantly increased the cell response to both IGF-I and des(1–3)IGF-I, whereas the addition of Pc significantly inhibited cell motility; * $P<0.01$. CTRL, control.

polymerization and membrane ruffling with protrusion of the cell leading edge in these cells (Kotani *et al.* 1994, Lauffenburger & Horwitz 1996, Leventhal *et al.* 1997). In SH-SY5Y cells, IGF-I is also known to stimulate lamellipodial advance and to promote tyrosine phosphorylation of paxillin and FAK, thus potentially playing a key role in tumour cell invasion (Leventhal *et al.* 1997).

Chemotaxis, a direct cell locomotion towards a soluble extracellular chemical gradient, is one of the steps necessary for migration of normal and aberrant cells. After binding of the chemokine to its receptor, various intracellular signals are generated, leading to a reconfiguration of the cytoskeleton, which is involved in the motile response. IGF-stimulated chemotaxis has been demonstrated in melanoma cells (Stracke *et al.* 1988), endothelial cells (Nakao-Hayashi *et al.* 1993), osteoblasts (Panagakos 1993), smooth muscle cells (Bornfeldt *et al.* 1994), breast cancer cells (Doerr & Jones 1996), and multiple myeloma cells (Vanderkerken *et al.* 1999). Understanding the biochemical events that govern the neuroblastoma metastatic spread is of major importance for controlling the disease. In addition, the cell line we used is considered a reliable model for neuronal development and the comprehension of the mechanisms underlying neuroblast motility might concur to clarify the pathophysiology of the congenital neuronal migration defects, such as agyria or lissencephaly.

In our migration assay, the truncated analogue des(1–3)IGF-I, which exhibits reduced binding capacity to IGFBPs, had a greater potency to stimulate SH-SY5Y cell migration than IGF-I. This finding strongly suggests that IGFBPs inhibit IGF-induced cell migration, locally modulating the IGF-I biological action. This inhibitory role of IGFBPs on IGF-stimulated cell migration has been described in smooth muscle cells (Gockerman *et al.* 1995) and, indirectly, in colonic epithelial cells (Andre *et al.* 1999). It is noteworthy that IGFBP-1 and IGFBP-2 contain the RGD (Arg-Gly-Asp) sequence which is required for binding to membrane integrins which mediate cell-extracellular matrix adhesion (Jones *et al.* 1993). Human neuroblastoma cells produce essentially IGFBP-2, smaller amounts of IGFBP-4, and traces of IGFBP-6 (Bernardini *et al.* 1994, Babajko & Binoux 1996, Cianfarani *et al.* 1996). IGFBP-2 is the major IGFBP in cerebrospinal fluid (Roghani *et al.* 1993), is produced in various regions of the central nervous system (Ocrant *et al.* 1990, Ishikawa *et al.* 1995) and, therefore, by binding to integrins and by modulating IGF actions may influence both cell adhesion and migration, thereby taking an active part in central nervous system fetal development and postnatal maintenance.

Most of the effects of IGF-I and -II are mediated by binding to type I IGF receptor (IGF-IR). Evidence that IGF-IR mediates the chemotactic response to IGF-I is provided by the finding that the monoclonal antibody, α -IR3, profoundly inhibited the IGF-I-stimulated cell motility. This inhibitory effect of α -IR3 on SH-SY5Y cell

migration is consistent with the observation that IGF-IR is a motility receptor in other cell lines such as melanoma cells (Stracke *et al.* 1989) and breast cancer cells (Doerr & Jones 1996), and its block inhibits the IGF-I-stimulated lamellipodial advance (Leventhal & Feldman 1997).

Binding of IGF-I to the extracellular α -subunits of the IGF-IR results in autophosphorylation of the cytoplasmic β -subunits which leads to a cascade of cellular signal transduction pathways (Van Obberghen 1994, White & Kahn 1994). One key event is the binding of insulin receptor substrate family (IRS-1 to -4) to phosphotyrosine residues on the receptor β -subunits (Avruch 1998). After binding by activated receptors, insulin receptor substrates are tyrosine phosphorylated and act as docking proteins for downstream signal transduction molecules, including PI-3 kinase and the Grb2-SOS complex (Kim *et al.* 1998a). Recent findings indicate that the PI-3 kinase pathway is implicated in IGF-induced membrane ruffling and growth cone extension, whereas the mitogen-activated protein (MAP) kinase pathway plays a key role in IGF-induced neurite outgrowth in SH-SY5Y cells (Waters & Pessin 1996, Kim *et al.* 1997). Our results show that PI-3 kinase inhibition significantly reduces the IGF-stimulated neuroblast migration, thus indicating that PI-3 kinase activation is required for neuroblast motility. However, the finding that PI-3 kinase inhibition reduces but does not abolish neuroblast migration suggests that when the main pathway (i.e. PI-3 kinase) is blocked, alternative pathways might be activated to maintain the IGF-stimulated cell migration. This hypothesis is consistent with the observation that PI-3 kinase inhibitors, such as wortmannin, increase tyrosine phosphorylation of IRS-2 and its association with Grb2 in SH-SY5Y cells (Kim *et al.* 1998b). However, the MAP kinase pathway is unlikely to be involved in this alternative regulation of neuroblast migration since PI-3 kinase inhibitors also cause a dose-dependent inhibition of MAP kinase pathway in neuroblasts (Kim *et al.* 1998b).

Plasmin plays a role in regulating cell proliferation and migration during embryonic development and in tumour invasion and metastasis, affecting cell adhesion to the extracellular matrix (Valinsky & Le Douarin 1985, Dano *et al.* 1985). Neuroblasts secrete tissue-type plasminogen activator (t-PA) which eventually induces the generation of plasmin (Neuman *et al.* 1989, Lalou *et al.* 1994) and, recently, plasmin-induced IGFBP proteolysis has been shown to enhance SH-SY5Y cell proliferation in response to IGFs (Menouny *et al.* 1997). Our results suggest that plasmin-mediated IGFBP proteolysis may increase IGF-induced neuroblast migration, thus indirectly confirming the role played by IGFBPs in modulating the cell response to IGFs and suggesting that the interaction between the IGF and plasmin system may play a pivotal role in regulating embryonic migration of neuroblasts and metastatic dissemination of neuroblastoma. However, it has to be pointed out that the addition of plasminogen also

increases basal (not significantly) and des(1–3)IGF-I-induced migration. At least three possible mechanisms may explain this finding: (a) plasminogen may enhance cell motility through a mechanism independent of IGFs and IGFBPs; (b) if plasminogen works specifically by degrading IGFBPs, its action in the absence of exogenous IGF-I must be due to the local secretion of IGFs; however, it remains unclear why plasminogen increases the des(1–3)IGF-I-stimulated migration to such a large extent; (c) the IGFBPs affected by plasminogen regulate motility in an IGF-independent manner.

In conclusion, IGF-I is a potent stimulator of neuroblast migration through the activation of type 1 IGF receptor and the PI-3 kinase intracellular pathway. IGFBPs and the plasmin system seem to play a role in cell motility although the nature and the extent of their involvement has yet to be elucidated.

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