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# COMMENTARY

# ROLE OF PROTEASE INHIBITION IN CELLULAR MIGRATION AND NEURITIC GROWTH

DENIS MONARD*
Friedrich Miescher-Institut, CH-4002 Basel, Switzerland

The integrity of the nervous system is required for its optimal function. A lesion of the peripheral nervous system is followed by regeneration phenomena which restore adequate function. Such regeneration phenomena do not take place following lesion in the central nervous system where the damage is irreversible. Experiments performed in recent years demonstrate that all neurons have the ability to regenerate if they are provided with the proper extracellular environment [1]. The existence of factors promoting neuronal survival and neurite extension has been demonstrated *in vitro* (for review see Ref. 2). Such factors have to be purified and characterized in order to study their relevance *in vivo*.

Cultured glioma and glial cells release a 43 kD protein which induces neurite outgrowth in neuroblastoma cells. This glia-derived neurite-promoting factor (GdNPF) has been purified to homogeneity, and we have demonstrated that it is a very potent inhibitor of serine protease activities which can be associated with the cell surface [3]. In addition to its neurite-promoting activity, purified GdNPF inhibits the migration of granule cell neurons monitored in cultured explants from early postnatal mouse cerebellum [4]. These results suggest that, during the development of the nervous system, the glial cells release GdNPF to regulate the migration of the neuroblasts and to create conditions compatible with neurite outgrowth. The discovery of GdNPF provided the first experimental evidence that cell-derived protease inhibitors play a role in cellular migration and neuritic growth. Recent results indicate that a protein similar or identical to GdNPF is detectable following lesion of the rat sciatic nerve but not following lesion of the optic nerve, suggesting that it could be involved in the regeneration phenomena which only take place in the peripheral nervous system [5].

*Protease inhibition in cellular migration*

The migration of cells is an important event taking place at specific locations and at specific stages during embryonic development. In most cases, the migrating cell is considered as an undifferentiated cell which will resume its differentiation programme only after having reached its final location. The cues regulating cellular migration and the subsequent steps of cellular differentiation are triggered by both genetic and epigenetic phenomena. The extracellular environment, therefore, has an important influence on these cellular events. The nature of the extracellular environment being distinct from location to location, the mechanisms which regulate the extent of cellular migration, and thus the final location of the cell, are of prime importance for the fate of each cell. Impairment of cell migration during development leads to irreversible, often lethal damage. For example, during the development of the cerebellum in the mouse mutant weaver, the migration of the granule cells is abortive or does not take place [6]. This leads to an anarchical organotypic organisation of the cerebellum and, thus, to premature death of the animal. Uncontrolled cellular migration, where the regulatory events fail to stop the invasive behaviour of the cell, is also deleterious. The case of metastasing tumor cells is certainly the most well known example. In such a pathological case, some characteristics that are required for cellular migration during development have escaped the control mechanisms.

Purified GdNPF inhibits the migration of the neuroblasts which leave the external granular layer to reach the internal granular layer at a specific stage of the development of the cerebellum [4]. This has been the first evidence that glial cells are able to release molecules which regulate the extent of the neuroblast migration and, therefore, have a decisive influence on the location of the immature neuronal cell. As each specific location provides a specific environment which will further monitor the subsequent steps of cellular differentiation leading to the mature functional neuron, glial cells have a key handle to influence the entire life span of some neurons. Obviously, more knowledge is required to understand the mode of action of GdNPF on this cellular migration. It is possible, however, to speculate and consider a working hypothesis which could motivate further experimentation.

Cellular migration, during normal development or during metastasis, is a dynamic event which requires repeated cycles of adhesion and deadhesion of the cell with its extracellular substratum. The migrating cells are following certain pathways which seem to be defined by an appropriate composition of the extracellular matrix [7]. The phase of adherence is certainly mediated by cell adhesion molecules (CAMs) which have been characterized at the cell surface [8]. Some of these CAMs are interacting with similar molecules located at the surface of neighbouring cells which could serve as a substrate(s) for

* Address all correspondence to: Dr. Denis Monard, Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

the migration. In fact, antibodies directed against L1, an antigen present at the surface of some neuronal cells, are able to inhibit the migration of granule cells which can be monitored in cultured explants of the developing mouse cerebellum [9]. Other cell surface associated molecules could be involved in the interaction of the migrating cell with specific components of the extracellular matrix such as fibronectin and laminin. *In vitro* experimental systems indicate that fibronectin and laminin, in fact, promote cellular migration [10, 11]. Moreover, a synthetic peptide representing the cell binding site of fibronectin blocks the cellular migration leading to the gastrulation at early developmental phases of the mouse embryo [12]. This elegant experiment indicates that the cell is no longer able to perform successfully this first phase of adhesion with the extracellular matrix if its fibronectin receptor is occupied by the synthetic peptide. It clearly demonstrates that interference with this phase of adhesion prevents migration. The cellular adherence can therefore be considered crucial for the guidance and the process of cellular migration. However, adhesion phenomena do not explain entirely the dynamic aspects of the migratory events. The adhesion phase has to be transitory and a subsequent phase of deadhesion is required to promote cell movement.

An increased amount of cell-associated proteolytic activity can be detected in migrating cells [13, 14]. It can be considered that this proteolytic activity is required to promote the phases of deadhesion and to allow the cell to penetrate the surrounding jungle of extracellular matrix components. The cell-associated proteolytic activity would therefore control the transitory nature of the interactions between the migrating cell and the extracellular supports which have to be loosened up or even destroyed. Some experiments, for example, have shown that fibronectin immunoreactivity disappears after the passage of migrating neural crest cells, suggesting an alteration, possibly a degradation of this important component of the extracellular matrix [7]. Although most of the data available today suggest an important role for fibronectin, other components of the extracellular matrix (collagen, laminin, vitronectin, thrombospondin, etc.) could also be involved in similar mechanisms required for the dynamics of the adhesion–deadhesion cycle.

Variations in the molecules involved in the adhesion–deadhesion cycle would strongly influence its turnover and, therefore, the speed of cellular migration. A decrease in the amount of cellular adhesion molecules or a locally reduced density of certain extracellular matrix components would break this cycle by interfering with the phase of adhesion. Alternatively, a reduction of the cell-associated proteolytic activity would automatically disadvantage the deadhesion phase in favour of the adhesion phase and therefore lead to a cessation of cellular migration. This could be achieved either by a reduction in the synthesis of the cell-associated proteolytic enzymes or by the production of inhibitors able to interfere with one or more of the proteases driving the deadhesion.

Cell-associated plasminogen activator is considered to be involved in such mechanisms [15]. High amounts of plasminogen activator activity are found in cells transformed with oncogenic viruses [16]. In the nervous system, this cell-associated proteolytic activity is enhanced at the developmental stage at which the migration of the granule neurons takes place [17]. The cells which invade the Bursa of Fabricius of quail and chick embryos also reveal increased plasminogen activator activity [18]. The function of this enzyme in cellular migration is not yet established but the use of specific antibodies against human urinary urokinase interferes with the invasion of human carcinoma cells injected in chick embryo, suggesting that this enzyme is one of the driving forces of the migratory events [19]. Plasminogen activators are today the only proteases that can be detected with some accuracy at the cellular level. It is therefore premature to conclude that they are the only proteases required for cellular migration, and it is quite feasible that other proteolytic enzymes with different substrate specificity will turn out to be as well needed or even more important.

The fact that glial cells release a macromolecular inhibitor able to form very tight complexes with different serine proteases does not exclude the possibility that other types of inhibitors with the same or a distinct specificity could also be released by different types of cells in order to slow down or even stop migrating cells.

*Protease inhibition in neurite outgrowth*

Is there some analogy between cellular migration and neuritic growth which could explain the neurite-promoting activity of GdNPF? It is generally assumed that the activity of the growth cone at the tip of the growing neurite is of key importance. Time-lapse cinematography studies illustrate the extreme motility of the filopodia of the growth cone. The filopodia are continuously extending and retracting. They are continuously going through phases of adhesion and deadhesion. One can thus consider the growth cone as a migrating subcellular structure of the differentiating neuroblast. An increased amount of plasminogen activator activity has been detected at the growth cone [20], suggesting that proteolytic processes are contributing here to the deadhesion phases required for the motility of the filopodia. However, the movements of the filopodia do not explain the net growth in neuritic length. In fact, neurite extension will only be promoted if a stabilization, contributing to a better adhesion, takes place with the modification of some "filopodial membrane" into "neuritic membrane". Such modification would not cause a depletion of growth cone structure since new material is transported anterogradely to be inserted continuously at the tip in order to form new "filopodial membrane". Protease inhibitors such as GdNPF, released by the surrounding glial cells, would interfere with the deadhesion phase of "filopodial membrane", possibly even contribute to its adhesion to the substratum, thus contributing to the changes leading to "neuritic membrane". Again, as for the inhibition of cellular migration, many biochemical events are probably involved in such changes. A high density of cellular adhesion molecules could, for example, be able to promote neurite outgrowth even without an important reduction in

the proteolytic activities associated with the growth cone. Alternatively, the inability of extracellular matrix to contribute to an appropriate adhesion could be deleterious to neuritic growth, even in the presence of sufficient amounts of cell-derived protease inhibitors.

In summary, as with cellular migration, a delicate balance between proteolytic activities, protease inhibitors, cellular adhesion molecules and some extracellular matrix components seems to be required for efficient neuritic growth. One can therefore also consider that each of the molecules involved has a chance to become rate limiting.

*Mode of action of cell-derived protease inhibitors?*

It is certainly plausible to think that GdNPF promotes neurite outgrowth and interferes with the migratory behaviour of the cells by inhibiting some cell-associated proteolytic activity. Protease inhibition however, does not seem to be a sufficient explanation. Other serine protease inhibitors have been tested for their effects on the migration of the granule neurons. None of them, including hirudin and a synthetic tripeptide which are able to promote extension in neuroblastoma cells at the same concentrations as GdNPF, interferes with these migrating neuroblasts. Moreover, hirudin and the synthetic tripeptide do not promote neuritic growth in primary neural cells cultured on components of the extracellular matrix. To explain these facts, one can postulate that the stable complex formed between the protease and GdNPF acquires properties that are not provided when the protease interacts with hirudin or the synthetic tripeptide. GdNPF, when complexed to the protease, could expose specific domains or epitopes which could promote an interaction between the cell and its surrounding, for example some components of the extracellular matrix. In this way, the inhibitor would have a double function: (1) to interfere with the deadhesion phase by blocking the function of the protease, (2) to strengthen the adhesion and efficiently contribute to the anchorage of the cell at the location where it has been stopped. Of course, such a model is presently only a working hypothesis which will have to be confirmed or invalidated by experimentation.

*Prospects*

GdNPF cDNA has been cloned recently.* The sequence of this cDNA has allowed us to deduce the amino acid sequence of the protein. With this knowledge, it is possible to chemically synthesize certain GdNPF epitopes or to produce longer GdNPF fragments by gene technology. Antibodies reacting specifically with such peptides and fragments can be raised. These new tools should provide opportunities to map the function of the different domains of GdNPF. They should allow scientists to establish whether the protease inhibitory activity is required, whether the complex formation with the protease reveals a GdNPF domain necessary for the interference with cellular migration, or whether GdNPF acts by a mechanism which does not even involve inhibition of proteolytic activity. Such an approach could, for example, allow one to define the GdNPF domain(s) which could bind to some components of the extracellular matrix and thus deprive the cell of the adequate interactions required for cellular migration or neurite outgrowth.

These tools should allow us to define the role and the importance of cell-derived high molecular weight protease inhibitors during axonal regeneration which takes place following lesion in the peripheral nervous system. They should thus provide opportunities to realize whether a lack of GdNPF or GdNPF-like proteins is an explanation for the abortive regeneration in the central nervous system. In such a case it would be of interest to study the influence of recombinant GdNPF on the kinetics of regeneration, both in the peripheral and in the central nervous system.

These tools should also allow us to study whether similar mechanisms can control the cellular migration taking place at early stages of embryonal life. The cDNA probes available could, in fact, serve to identify similar but distinct cell-released protease inhibitors which could have distinct organ localization and act at specific stages during development.

The first 28 residues of GdNPF are identical with the aminoterminal sequence of protease-nexin I determined by chemical means [21].* The data support the conjecture that GdNPF belongs to the protease-nexin family. It is, in fact, the first member of this family whose sequence is completely known. Protease-nexins being inhibitors secreted by different types of cells [21, 22], one can consider that such a mechanism regulating cell migration is not restricted to the nervous system.

It would, in fact, be of very special interest to study whether metastasizing tumor cells use similar adhesion–deadhesion mechanisms to migrate and invade tissues. If this were the case, it would be of prime importance to study the potency of such cell-derived inhibitors on the migration of tumor cells. Obviously cellular migration is a multifunctional phenomenon where, beside proteases and protease inhibitors, other enzymes released by the migrating cell, other cell surface components, or different components of the surrounding extracellular matrix could turn out to be of crucial importance. Nevertheless, an understanding of these mechanisms should enable scientists to estimate how much they are interrelated and which of them becomes rate limiting at different stages of cellular migration. With such basic knowledge available, one could then consider that the new methods offered by modern gene technology will lead to new tools able to reduce or block pathological cellular migration.

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