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From elastin peptides to neuraminidase-1-dependent lactosylceramide generation

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ABSTRACT

Elastin peptides constitute a group of biologically active peptides derived from the fragmentation of insoluble elastin. These molecules, currently termed elastokines, have been shown to interact preferably with the elastin receptor complex. Recent data show that the sialidase activity of the neuraminidase-1 of this receptor is required for these peptides to induce their effects. As lactosylceramide generated at the plasma membrane by desialylation of the ganglioside GM_3 can be considered as a second messenger, we feel that this lipidic compound could mimic elastin peptides effects in physiopathological situations.

RÉSUMÉ

Les peptides d'élastine constituent un groupe de peptides biologiquement actifs provenant de la fragmentation de l'élastine insoluble. Ces molécules, aujourd'hui appelées élastokines, interagissent préférentiellement avec le complexe récepteur de l'élastine. Des données récentes montrent que l'activité sialidase de la sous-unité neuraminidase-1 de ce récepteur est requise pour que ces peptides induisent leur effet. Comme le lactosylcéramide produit à la membrane plasmique par désialylation du ganglioside GM_3 peut être assimilé à un second messager, nous pensons que ce composé lipidique pourrait mimer les effets de peptides dans des situations physiopathologiques.

The resilience of vertebrate tissues is due to the presence of elastic fibers in the extracellular space [1]. These structures are more abundant in tissues where resilience is required, such as skin, lung, ligaments or large arteries. Their principal and central component is elastin, an extracellular matrix protein [2]. It is an insoluble, amorphous, hydrophobic and extensively cross-linked protein present in variable amounts depending on the tissue [1].

In most tissues, elastin production begins at mid-gestation and peaks at birth. It then drops dramatically and is nearly completely repressed by maturity [3]. The hydrophobic and highly cross-linked nature of elastin makes it a very durable polymer, resistant to enzymatic proteolysis and experiencing essentially no turnover in healthy tissues. As elastin is a long-lived protein [4], it is assumed that an individual has his elastin capital by teenage. After that critical point, elastin begins to be degraded into fragments following the action of elastases. Elastin fragmentation is one of the hallmarks of ageing [5].

Elastin degradation products, the so-called elastin peptides (EP), have long been thought to be mere waste bound to be disposed off by the organism. But, in the early

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1980s, it was evidenced that tropoelastin and proteolytic fragments of insoluble elastin displayed potent chemotactic activity for human monocytes and fibroblasts [6]. Since that pioneering work, numerous authors have shown that EP obtained from alkaline (κ-elastin [kE]) or enzymatic hydrolysis of polymeric elastin could modulate several cellular functions *in vitro* that could be associated to severe age-related diseases [7]. Notably, the works dealing with EP effects and their signaling established that these compounds could modulate ions fluxes in mononuclear cells, fibroblasts and smooth muscle cells [8–10], and that the chemotactic activity of these peptides relied on an intracellular increase of Ca^{2+}, AMPc and/or GMPc leading to the activation of various kinases such as PKA, PKG or PKC depending on the considered cell type [11–14].

Our group has significantly contributed to this effort by focusing its research on the modulation of matrix metalloproteinase (MMP) levels by EP, notably kE. In the subsequent text, we will explain the research path we followed from EP to glycosphingolipids.

Our founding paper was published in 2001 [15]. In that contribution, we showed that elastin hydrolysates as well as synthetic elastin sequences could promote the accumulation of important levels of pro-collagenase-1 (pro-MMP-1) in the culture medium of human skin fibroblasts. MMP-1 is one of the rare protease able to degrade fibrous collagen and, as a consequence, the fact that EP could promote its synthesis was an important finding suggesting that elastin fragmentation could somehow lead to collagen breakdown. Using synthetic peptides, we were also able to show that the consensus sequence recognized by cells was GXXPG, a pattern thought to promote the formation of type VIII β-turn.

At that time, the nature of the elastin receptor was strongly discussed. The elastin receptor complex (ERC) was described as a heterotrimer related to the lysosomal β–galactosidase complex. It was observed at the surface of numerous cell types [16] and comprised a peripheral 67 kDa subunit which actually binds elastin, the elastin binding protein (EBP), a 55 kDa protective protein, and a 61 kDa membrane-bound neuraminidase [17]. Since the very nature of the ERC was still discussed, the way this receptor transduced signals was largely unknown. As a consequence, we chose to analyze the late signaling events induced by EP binding to EBP with the hope of finding clues about the nature of early signals.

According to the established fact that MMP-1 promoter activity is highly dependent on AP-1 signaling, we showed [18] that EP-triggered EBP signaling involved the rapid activation of the MEK/ERK cascade, ultimately resulting in AP-1-dependent transcription of the MMP-1 gene. Further [19], we evidenced that this activation originated from the simultaneous involvement of the G protein/p110γ/Raf-1 and cAMP/PKA/B-Raf signaling pathways leading to a strong and sustained activation of MMP-1 transcription.

At that point, our signaling scheme had led us to the plasma membrane and we had no real clues about how the signal could be initiated by the ERC. Fortunately, in 2006, Aleksander Hinek demonstrated that the neuraminidase involved in the ERC was neuraminidase-1 [20]. This finding made the link with former data showing that this lysosomal sialidase could also be targeted to the cell surface when its cytoplasmic tail was phosphorylated [21].

These data led us to reconsider our view of the receptor. Indeed, at that time, it was conventionally accepted that the membrane-bound subunits of the receptor were catalytically inactive. Hinek's work elegantly demonstrated that it was not true. As a consequence, we stimulated our cells with EP in the presence of specific inhibitors for the protective protein cathepsin A activity and for neuraminidase-1 sialidase activity. These experiments, as well as the use of siRNA, permitted us to demonstrate that Neu-1 sialidase activity was an absolute requirement for proper EP signaling and further induction of the ERK 1/2 pathway [22]. However, at that time, we were not able to identify the substrates desialylated by Neu-1.

Neu-1 is a member of the sialidase family and catalyzes the removal of sialic acids from the sugar chains of glycoproteins and glycolipids [23,24]. Seyrantepe et al. [24] have shown that GM_3 ganglioside is a substrate of Neu-1. Gangliosides are sialic acid-containing glycosphingolipids found in the outer leaflet of the plasma membrane of vertebrate cells [25]. They are involved in cellular interactions and signal transduction [26]. In membranes, gangliosides are found in lipid rafts, highly organized plasma membrane microdomains enriched in cholesterol, glycosphingolipids and transmembrane proteins [27] that are important signaling platforms [27,28].

Lactosylceramide (LacCer) is derived from the GM_3 ganglioside precursor. It is involved in fibroblast proliferation [29], ERK 1/2 activation in smooth muscles cells [30] and angiogenesis [31]. Interestingly, all of these effects can be triggered by EP [32].

Therefore, we made the hypothesis that EP effects could originate from Neu-1-mediated glycosphingolipid conversion occurring in lipid rafts. Indeed, after having shown that the receptor and lipid rafts colocalized at the plasma membrane, we established that the disruption of these microdomains blocked the receptor signaling, as did their depletion in glycolipids. Further, we observed that cellular GM_3 levels decreased following EP treatment, while LacCer one increased consistently with a GM_3/LacCer conversion. Finally, we readily demonstrated the importance of Neu-1 in this conversion as the use of a Neu-1 siRNA or of a monoclonal anti-GM_3 blocking antibody blocked this process [33].

After 10 years of research, we now obtain some answers. EP effects are born in the membrane itself, more precisely in lipid rafts. Undoubtedly, we will have to find the answers to our future questions in these regions. The fact that LacCer is an early messenger of the ERC let us foresee new approaches using this compound in pathological or pharmacological situations.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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