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Beyond the cell surface: New mechanisms of receptor function

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ABSTRACT

The text book view of cell surface receptors depicts them at the top of a vertical chain of command that starts with ligand binding and proceeds in a lineal fashion towards the cell nucleus. Although pedagogically useful, this view is incomplete and recent findings suggest that the extracellular domain of cell surface receptors can be a transmitter as much as a receiver in intercellular communication. GFRα1 is a GPI-anchored receptor for GDNF (glial cell line-derived neurotrophic factor), a neuronal growth factor with widespread functions in the developing and adult nervous system. GFRα1 partners with transmembrane proteins, such as the receptor tyrosine kinase RET or the cell adhesion molecule NCAM, for intracellular transmission of the GDNF signal. In addition to this canonical role, GFRα1 can also engage in horizontal interactions and thereby modify the function of other cell surface components. GFRα1 can also function as a ligand-induced adhesion cell molecule, mediating homophilic cell–cell interactions in response to GDNF. Finally, GFRα1 can also be released from the cell surface and act at a distance as a soluble factor together with its ligand. This plethora of unconventional mechanisms is likely to be a feature common to several other receptors and considerably expands our view of cell surface receptor function.

1. Vertical signaling by GDNF receptor complexes

Neurotrophic factors are key regulators of neuronal survival, differentiation, migration synapse formation and function in both the developing and adult nervous systems. GDNF (glial cell line-derived neurotrophic factor) was originally isolated from the supernatant of a glial cell line based on its effects on the survival of midbrain dopaminergic neurons, those that degenerate in Parkinson's disease [1]. It was later found that GDNF has effects on many other types of neurons in both the peripheral and central nervous systems [2]. The first GDNF receptor to be identified was RET, a receptor tyrosine kinase that had been found mutated in endocrine human cancers and cases of Hirschsprung disease [3]. Although required for GDNF function, RET was found to be unable to bind the factor on its own, leading to the discovery of the GFRα1 (GDNF family receptor alpha 1) subunit through expression-cloning efforts [4]. GFRα1 is anchored to the plasma membrane via a GPI link and hence lacks transmembrane and intracellular domains. Three other factors with sequence homology to GDNF were subsequently identified, termed Neurturin, Artemin and Persephin, respectively [2]. Although RET mediates the signaling of all members of the GDNF family, three additional GPI-linked co-receptors related to GFRα1, termed GFRα2, 3 and 4, were found to specifically bind each of the newly identified family members [2]. In the mammalian brain, expression of GFRα1 and 2 is more prominent, while GFRα3 and 4 are predominantly found in the peripheral nervous system and other organs, respectively. The GDNF/GFRα1/RET complex activates many of the signaling pathways that have been characterized in other receptor tyrosine kinases, such as the Ras/MAPK, PI3K/Akt and PLCγ pathways [5] (Fig. 1A), and several recent studies have begun to dissect the importance of each of these pathways for the neurotrophic actions of this receptor complex [2].

Since GFRα1 lacks an intracellular domain, the fact that this receptor appeared expressed in many brain areas in the absence of RET was taken as an indication that additional signaling receptors might exist [6]. Several intracellular events elicited by GDNF were subsequently described in cells expressing GFRα1 but lacking RET [7]. Together with chemical cross-linking studies, these insights led to the discovery of the neural cell adhesion molecule NCAM as an alternative signaling receptor for GDNF and related molecules [8]. NCAM had been known for its ability to mediate cell adhesion as well as a handful of downstream signaling events triggered by homophilic NCAM interactions [9]. Unlike RET, however, NCAM was found to be able to bind GDNF directly, but high-affinity binding and downstream signaling required the presence of GFRα1 [8], and molecular modeling and site-directed mutagenesis studies revealed that GDNF specifically interacts with the third Ig domain of NCAM [10,11]. An NCAM mutant has been generated that is unable to bind GDNF but which still retains the ability to mediate cell adhesion [10], indicating that the two functions can be genetically separated. Downstream signaling by NCAM in response to GDNF has been shown to involve events similar to those elicited

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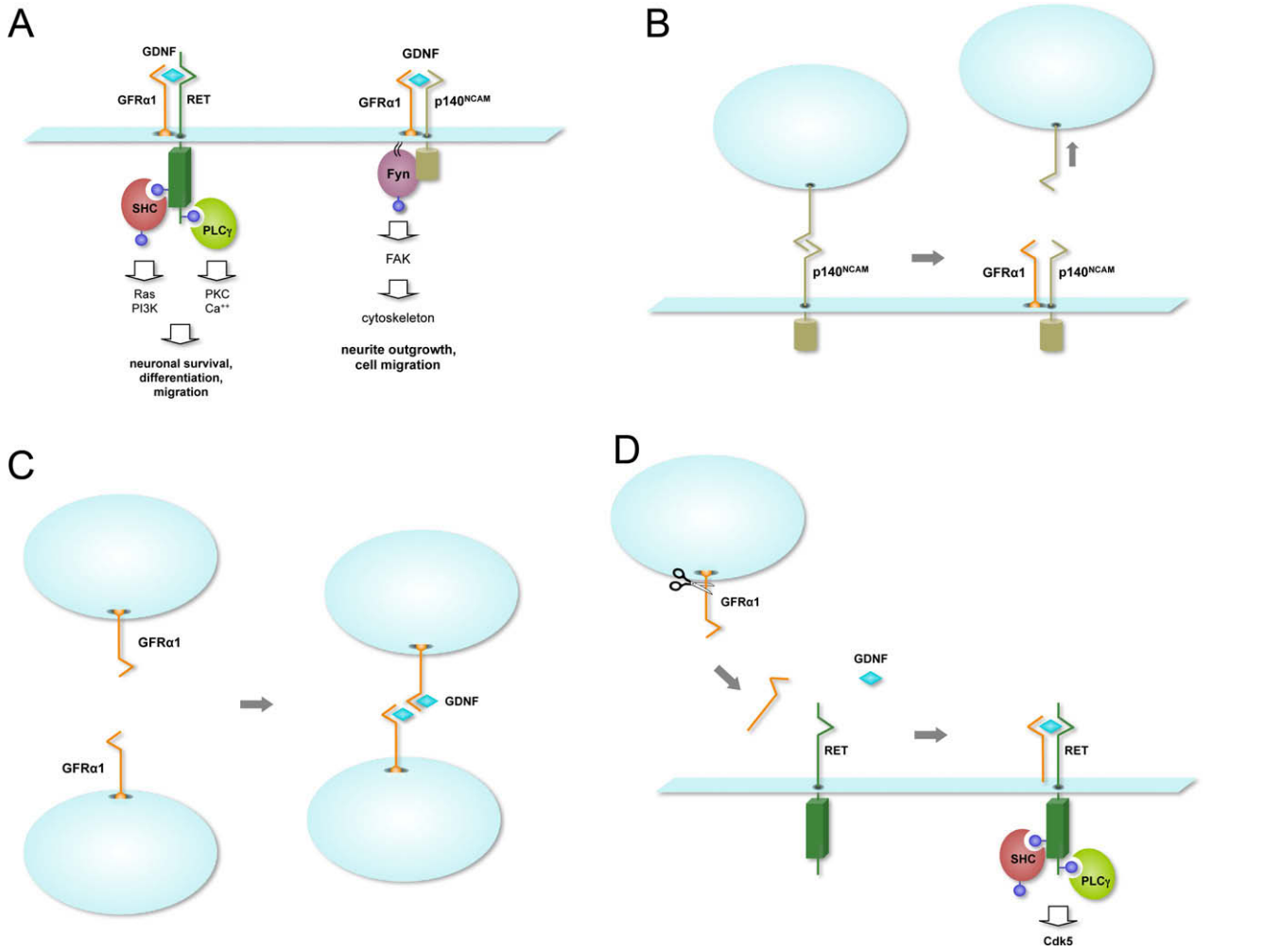

Fig. 1. Multitasking in GFRα1 receptor function. (A) Vertical signaling together with RET or NCAM. (B) Horizontal interactions downregulate NCAM-mediated cell adhesion. (C) Ligand-induced cell adhesion. (D) Non-cell-autonomous action at a distance.

by homophilic interactions, including activation of Fyn kinase [8] (Fig. 1A). Through the NCAM/GFRα1 complex, GDNF has been shown to regulate cell migration, neuronal morphology and synapse formation [8,11–13].

2. Horizontal interactions regulate NCAM-mediated cell adhesion

In addition to its classical role as a GDNF receptor, GFRα1 also engages in horizontal interactions that affect the function of other cell surface components. As reviewed above, GFRα1 has been shown to form a complex with NCAM, and this interaction has been thought to be important for GDNF high-affinity binding and downstream signaling through the GFRα1/NCAM complex [8]. One intriguing effect of the binding of GFRα1 to NCAM is the downregulation of NCAM's ability to mediate cell adhesion [8] (Fig. 1B). Even low relative levels of GFRα1 can cause NCAM to be significantly less competent at triggering homophilic cell–cell interactions, an effect that it is independent of the presence of GDNF. Binding of GFRα1 to NCAM presumably masks important determinants for homophilic NCAM interactions. Because GFRα1 and NCAM are co-expressed in several populations of migratory neuronal precursors in the developing and adult brain, such as those in the rostral migratory stream (RMS), this finding may have important implications for the control of cell migration through regulation of the cell adhesion activity of NCAM [14].

The interaction between GFRα1 and NCAM is direct, and more recent work has shown it to be mediated by the N-terminal domain of GFRα1 and the fourth Ig domain of NCAM [15]. The N-terminal domain of GFRα1 does not participate in GDNF binding, but it is both required and sufficient to inhibit NCAM-mediated cell adhesion [15], a finding that identifies the first known function for this region of the GFRα1 molecule. Unfortunately, current three dimensional models of GFRα molecules based on the crystal structures of the GFRα1/GDNF and GFRα3/Artemin complexes lack any atomic information on the N-terminal domain, so additional studies will be required to shed light on this important region of the GFRα family of molecules. Intriguingly, the N-terminal domain of GFRα1 is dispensable for the ability of GFRα1 to potentiate GDNF binding to NCAM [15]. Together these data indicate that direct receptor–receptor interactions are not required for high affinity GDNF binding to NCAM but play an important role in the regulation of NCAM-mediated cell adhesion by GFRα1. They illustrate how even relatively small cell surface receptors can have diverse functions at the cell membrane, i.e. through both vertical and horizontal interactions, via different regions of their extracellular domains.

3. Ligand-induced cell adhesion

An intriguing function of GFRα1, not directly related to its ability to mediate intracellular signaling, was accidentally discovered

during the course of investigations on the trophic activities of GDNF in cultures of hippocampal neurons. GDNF was shown to induce the formation of synapses in those cultures, an effect that required the expression of GFRα1 on the neurons [13]. Biochemical and immunocytochemical studies helped to establish the presence of the receptor at both the pre- and post-synaptic sides of hippocampal synapses. The realization that many other synaptic cell surface molecules that contribute to synapse formation, such as Neuroligins/Neurexins, DSCAM and Ephrins/Ephs, also have the ability to mediate cell adhesion, led to investigations of the adhesive properties of GFRα1. Although the receptor was by itself not able to mediate cell adhesion in standard cell aggregation assays, it was found that addition of GDNF induced a robust and long-lasting response, promoting the formation of large cell clusters similar to those typically observed upon overexpression of *bona fide* cell adhesion molecules, such as NCAM [13]. Because cell adhesion was only observed after addition of the GDNF ligand, the term ligand-induced cell adhesion (LICA) was coined to differentiate this novel process from other more traditional mechanisms of cell–cell interaction in which cell adhesion is a direct, default consequence of the mere expression of cell adhesion molecules at the cell surface (Fig. 1C). LICA uniquely combines the features of soluble and cell-associated molecules to mediate cell adhesion in a way that becomes amenable to exquisite regulation by either the participating cells themselves—in a cell-autonomous fashion—or standby cellular components in a non-cell-autonomous way through the production and release of the GDNF ligand. There are no other examples known of ligand-induced cell adhesion molecules (LICAMs) but it is possible that many other cell surface receptors function in this way.

Importantly, LICA was not simply a consequence of GDNF being a dimer and GFRα1 being able to bind GDNF in dimeric form. Other receptors known to interact with dimeric ligands as receptor homodimers, such as the NGF receptors TrkA and $p75^{NTR}$, are not able to function as LICAMs ([13] and unpublished results). This would argue against a simple trans-homodimerization mechanism with the ligand functioning as a "glue" between the interacting cells, although it remains possible that unique features of the GDNF/GFRα1 complex may allow this to happen. Another possibility is that GDNF induces some kind of conformational change in GFRα1 that exposes receptor determinants able to mediate homophilic cell–cell interactions. However, recent crystallographic studies of the GDNF/GFRα1 complex do not support the existence of such a conformational change [16]. Moreover, and although the GFRα1 N-terminal domain was not present in the crystal structure of the complex, its deletion does not affect the LICAM activity of GFRα1 [13]. If neither GDNF nor GFRα1 are directly responsible for cell–cell contact, it remains possible that additional components, such as for example extracellular matrix elements, may play a role. Regardless of the mechanism, the LICA phenomenon has emerged as a novel way in which cell–cell interactions can be formed and regulated, and has important implications for a myriad of morphogenetic events including cell migration, axon growth and synapse formation.

4. Non-cell-autonomous effects: soluble GFRα1 acts at a distance

As with most GPI-anchored receptors, GFRα1 is efficiently shed from the cell surface in a constitutive manner by the action of membrane-associated phospholipases. Soluble GFRα1 binds GDNF with high affinity, is very stable, and can be readily recovered from the supernatant of GFRα1-expressing cell lines and tissues, such as lesioned peripheral nerves [17]. Several studies have shown that released GFRα1 can act at a distance to potentiate downstream signaling, neuronal survival and neurite outgrowth in response to GDNF [17,18]. The discovery that peripheral targets of innervation of sensory and sympathetic axons express high levels of GFRα1 prompted the study of potential axon-guidance activities of GFRα1 released by target cells. It was found that soluble GFRα1 could capture GDNF, present it to axonal RET receptors and thereby induce axon growth in a directional fashion [18] (Fig. 1D). Target-derived GFRα1 could thus function as a long-range directional cue for growing axons [19]. These effects were found to be mediated by activation of the Cdk5 kinase, which had previously been linked to neuritic growth and axon guidance. Interestingly, the roles of Cdk5 in GFRα1-mediated growth and guidance could be dissociated. Specifically, it could be shown that higher levels of Cdk5 activity are required for directional responses to exogenous GFRα1 which were sensitive to low levels of Cdk5 inhibitors [18].

A feature shared by GPI-anchored proteins is that they can be found in either membrane bound or soluble forms. Secretion of biological, active GFRα1 by known targets of RET-expressing neurons may form diffusible gradients that could promote long-range effects on developing axons. On the other hand, GPI-anchored GFRα1 expressed on stand-by cells can also capture and present GDNF in trans to RET receptors on adjacent cells [17]. Thus, GFRα1 has the capacity to elicit both long-range and localized guidance effects by creating positional information for RET-expressing axons even in the presence of a uniform concentration of GDNF. GFRα1 can thereby rearrange GDNF into a spatial pattern that may be different from the initial pattern of GDNF gene expression. These results have unraveled a novel role for cell surface receptors through an ability to act at a distance, non-cell-autonomously, on other cells. A mutant mouse generated to test some of these ideas failed to show an effect following deletion of GFRα1 from selected groups of target tissues [20]. Further investigations of the *in vivo* relevance of non-cell-autonomous activities of GFRα1 will require novel animal models carrying altered versions of the GFRα proteins.

5. Concluding remarks

Studies on the structure and function of the GFRα1 protein have revealed a wider functional landscape than what was initially anticipated. Cell surface receptors can not only function in a traditional—vertical—fashion by binding an extracellular ligand and mediating signals across the plasma membrane, but also engage in a number of alternative interactions, both in the plane of the membrane and beyond (Fig. 1). The examples described here illustrate the many ways in which intercellular communication can take place and extend the classical concept of cell surface receptor to one encompassing functions that were either unknown to exist or thought to require dedicated groups of molecules.

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