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Cell Communication Insights

Contact-dependent Signaling

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ABSTRACT: It is becoming increasingly clear that communication between cells is carried out not only by the signaling molecules themselves, but also by many contextual and positional cues that arise from the way the signal is distributed and presented to the receptor. Many cells express transmembrane growth factors that use their extracellular domain for signaling to cells connected by adhesion. Some of these growth factors can also be receptors for a reverse signal from the adhesion partner. Secreted growth factors or their receptors can engage in contact-dependent signaling by associating with extracellular matrix (ECM) components and integrins. Signaling molecules can also reach cells at a distance via cytonemes that contact and activate the target cell through synapse-like structures.

KEYWORDS: contact-dependent signaling, juxtacrine, paracrine, autocrine, semaphorin, ephrin, notch, extracellular matrix, heparan sulfate, netrin, integrins, tetraspanin, cytonemes

CITATION: Denef. Contact-dependent Signaling. *Cell Communication Insights* 2014:6 1–11 doi:10.4137/CCI.S13924.

RECEIVED: August 26, 2014. **RESUBMITTED:** October 21, 2014. **ACCEPTED FOR PUBLICATION:** October 24, 2014.

ACADEMIC EDITOR: Paul J. Higgins, Editor in Chief

TYPE: Review

FUNDING: Author discloses no funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Paper subject to independent expert blind peer review by minimum of two reviewers. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE). Provenance: the authors were invited to submit this paper.

Introduction

Multicellular organisms have developed elaborate biochemical and biomechanical communication networks in order to faultlessly orchestrate their reproduction and development, and to maintain integrity during adult life.[1,2] Communication needs signals, receptors, and a structural organization that embeds cells in their specific locations and guides the signals to their targets. Signaling occurs between cells, between the extracellular matrix (ECM) and cells, and between subcellular compartments. Communication is therefore an essential constituent of multicellular organisms.

Biochemical signals range from small molecules such as ions, gases, amino acids, monoamines, purines, pyrimidines, retinoids, fatty acids, lipids, and steroids to peptide, protein, and nucleotide macromolecules.[3] Signaling systems are evolutionarily conserved but both signals and receptors are highly diversified during evolution. Diversity also stems from differential mRNA splicing, posttranslational modifications by polysaccharides, heparan sulfate (HS) and chondroitin sulfate, cholesterol and lipids, receptor hetero-oligomerization, and specific association of ligands and receptors with extracellular, transmembrane, and intracellular proteins. Responses to signals depend not only on their chemical nature, dose, and signal transduction system used, but also on their temporal and positional appearance. In addition, signals can be stored in the ECM, and variations in constituents of the ECM can create different contexts that co-determine signaling outcome.[1,2]

Several modes of intercellular signaling have been distinguished on the basis of how and how far the signal travels and how it is presented to the target cell.[4] Signals can be released by cells and move either to distant or to neighboring cells. A signal transported by the blood to remote target cells is called a *hormone*. When the signaling molecule acts on cells in the vicinity, it is called a *paracrine* factor. If the responding

cell is the emitting cell itself, the signal is called an *autocrine* factor. Many paracrine and autocrine systems have been discovered,[3] but it remains underexplored how and how far these substances distribute once released in the extracellular space.[5]

Signaling can also require close physical contact between emitting and receiving cells[5] or between the ECM and cells.[6] Direct cell–cell contact enables membrane-anchored ligands on the emitting cell to reach their cognate receptors on the receiving cell. It is known as *juxtacrine* communication. The ECM can profoundly affect signaling of secreted molecules by associating with receptors and/or ligands or keeping the ligands tightly localized near their target receptor. Signaling can also occur toward specified cells at a distance via very thin and long cytoplasmic extensions, known as *cytonemes*,[7] very much like signaling by neurotransmitters released within the *neuronal synapse*. Signaling between antigen-presenting cells and T cells through the so called *immunological synapse* also requires adhesive contact between the antigen peptide and the T cell receptor.[8] Contact-mediated communication can also occur through specialized junctions, known as *gap junctions*, which allow direct transfer of small molecules (molecular weight <1500) and ions from the cytoplasm of one cell to that of its neighbor.[9]

The present review will deal with the growing awareness that cell–cell and cell–matrix contacts play an essential role in intercellular communication and will review the molecular architecture underlying these phenomena. For more specific topics related to signaling in the neuronal and immunological synapses, the reader is referred to recent reviews.[8,10]

Juxtacrine Signaling by Membrane-anchored Growth Factors

This mode of communication was first described by Massagué's group, who discovered that mouse bone marrow stromal cells in co-culture with hematopoietic progenitor cells do not signal through the soluble transforming growth factor-α (TGFα) to the epidermal growth factor (EGF) receptor (R) on the progenitor cells, but through the membrane-bound TGFα precursor.[5] It was also observed that this mode of EGFR activation induced adhesion between the two cell types. Since then, a similar signaling mode has been demonstrated for other members of the EGF family, members of the tumor-necrosis factor (TNF) superfamily, immunomodulators, various interleukins, certain chemokines and hematopoietic factors, Notch ligands, ephrins, semaphorins, and certain netrins (Table 1).[5,11–40] Juxtacrine signaling has also been shown in invertebrates, such as by lin-3 in *Caenorhabditis. elegans*,[41] and bride of sevenless (boss) in *Drosophila*.[42]

Although most juxtacrine signals are type I transmembrane proteins, some are type II, such as the TNF superfamily members. Some are bound to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor, such as A-ephrins,[43] bone marrow stromal cell antigen 1 (BST1),[40] and semaphorin 7.[30] Interleukin 1 may also be anchored to the extracellular side of the plasma membrane by a lectin or a mannose receptor.[44]

Table 1. Overview of transmembrane signaling molecules.

MOLECULES	REFERENCE
Epidermal growth factor (EGF) family	
Transforming growth factor α (TGFα)	5
Amphiregulin	11
Heparin-binding EGF (HB-EGF)	12
Betacellulin	13
Neuregulins	14
Tumor necrosis factor (TNF) superfamily	
TNF-α	15
CD27 ligand	16
CD30 ligand	17
CD40 ligand	18
TNF-like weak inducer of apoptosis (TWEAK)	19
Fas ligand	20
Receptor activator of nuclear factor κB ligand (RANKL)	21
Interleukins (IL)	
IL1	22
IL2	23
IL15	24
Hematopoietic factors	
Macrophage colony-stimulating factor (M-CSF)	25
Colony-stimulating factor (CSF-1)	26
Kit ligand	27
Ephrins	28
Notch ligands	29
Semaphorins	30
Netrins	
UNC-6/Netrin bound to its receptor UNC-40/DCC	31
Netrin G1 and G2	32
Nogo proteins	33
Desert Hedgehog	34
Immunomodulators	
CD93	35
Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)	36
Betaglycan	37
Chemokines	
CX3CL1	38
CXCL16	39
Bone marrow stromal cell antigen 1 (BST-1, CD157)	40

In juxtacrine signaling, the spatial distribution is extremely small as compared to that of hormone, paracrine, and autocrine signals.[4] Once the adhesive contact is made, the ligand is supposed to associate with the nearby receptor in a non-equilibrium fashion, but how the necessary cell–cell

adhesion exactly occurs remains underexplored. In contrast, the spatial distribution of a hormone is systemic and uniform, and the response depends on the number of receptors in equilibrium with the ligand. The action of juxtacrine signals is restricted to only those cells that are in direct contact with the emitting cells, as only through adhesive contact the receptors are juxtaposed closely enough to allow interaction between the membrane-anchored signal and the receptor. These cell associations also ascertain selective targeting of cells excluding interaction of the signal with the same receptor on other cell bodies in the vicinity.

The default signaling mode by hormones and most paracrine and autocrine systems is from ligand to receptor (forward signaling), but juxtacrine signaling can be bidirectional ie from receptor to ligand as well (reverse signaling). Ligands acting as receptors include ephrins,[45] macrophage colony-stimulating factor (M-CSF),[46] BST1,[40,47] Notch ligands,[48] IL15,[24] neuregulins,[49] semaphorins,[30,50] and members of the TNF superfamily.[51]

A few juxtacrine signaling systems are discussed in more detail below.

Semaphorins. (Fig. 1) semaphorins were originally discovered as repulsive axon guidance molecules,[30] but were subsequently found to have significant roles in many other tissues and to play a role in cancer progression.[50] Semaphorins operate either as transmembrane proteins (SEMA1, SEMA4, SEMA5, and SEMA6 members), GPI-anchored proteins (SEMA7A), or as secreted molecules (SEMA2 and SEMA3 members). Most semaphorins bind to their high affinity plexin receptors directly, while SEMA3 members require, in addition, neuropilin-1 or -2 as co-receptor. Some SEMA3 members may signal independently of plexins through immunoglobulin cell adhesion molecules (IgCAM), while SEMA7A signals through neuronal integrin receptors and plexin C1 receptor.

A peculiar characteristic is that plexins can associate with several other receptors, including other plexins and the receptor tyrosine kinases Met, ERBB2, and vascular endothelial growth factor receptor (VEGFR), to form multimeric complexes, which provide plexins access to the transduction pathways of the binding partner (reviewed in ref. 30). Furthermore, SEMA5 signaling is differentially modulated by heparan sulfate proteoglycans (HSPGs) and chondroitin sulfate proteoglycans (CSGPs). SEMA5A attracts axons expressing HSPGs via a hitherto unidentified receptor, while it repels axons expressing CSGP.[30] All these different co-receptor

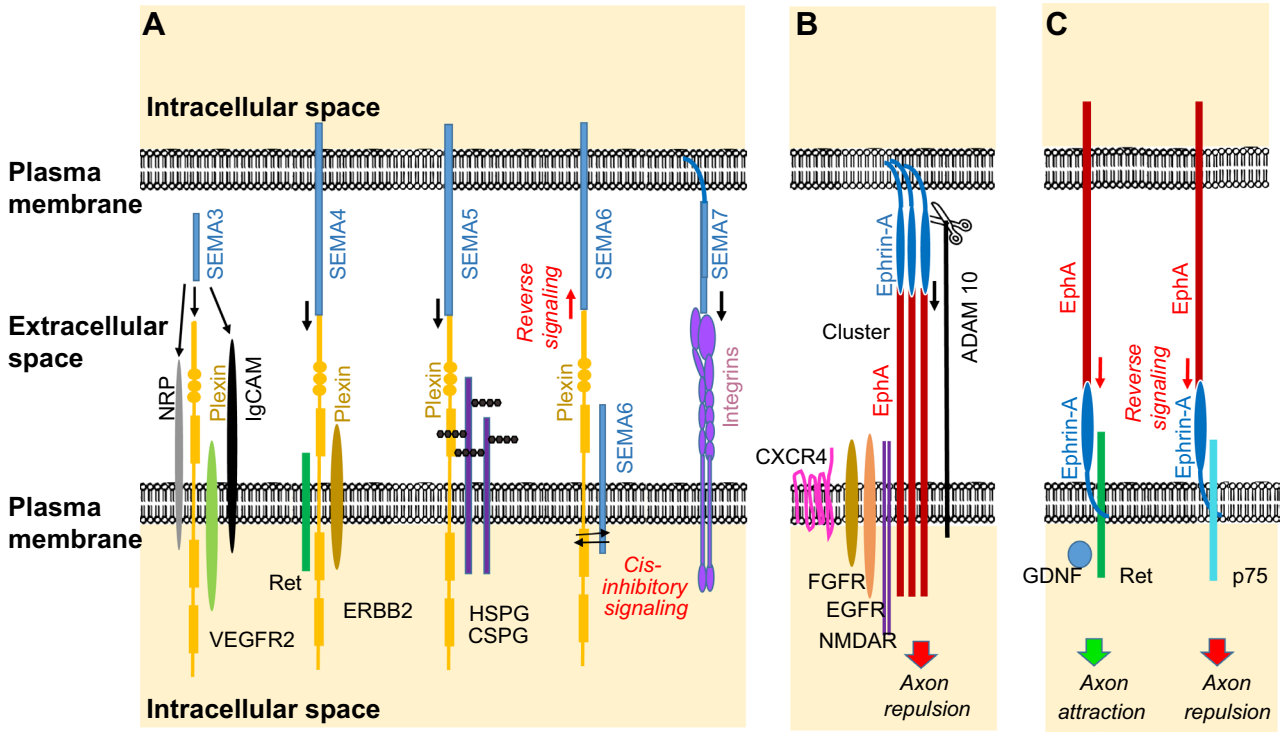

Figure 1. Simplified overview of the different modes of juxtacrine signaling by semaphorins and ephrins. (**A**) Semaphorins are either soluble or transmembrane or GPI-anchored proteins. Many SEMAs signal through specific plexins. Some SEMAs form signal through IgCAM, NRP, or integrins. Plexins can associate with other receptors such as ERBB2 and VEGFR2, and with HSPGs and CSPGs, which modulates signal transduction and biological outcome. Plexins can reverse-signal to SEMA6 and engage with SEMA6 in cis-inhibitory signaling. (**B**) Ephrins and Ephs make oligomeric clusters to allow signal transduction. A cluster of Ephs can incorporate other Ephs as well as other tyrosine kinase receptors such as FGFR, EGFR, NMDAR, and CXCR4, leading to modulation of the signal transductions. ADAM 10 expressed in the Eph-bearing cell also associates with the Ephrin–Eph cluster and terminates signaling by cleaving off the ligand extracellular domain. In neurons, ephrin-bearing cells usually induce repulsion of the Eph-expressing axon. (**C**) Eph can also function as a reverse signal to the ephrin-bearing cell. This occurs for example when the GDNF receptor Ret associates with an ephrin-A. This results in axon attraction, which is synergistically stimulated by soluble GDNF.

interactions create high diversity in response. Another peculiarity is that semaphorins can also act as receptors for plexins, resulting in reverse signaling.[30]

Ephrins. (Fig. 1) another striking juxtacrine signaling system is that between ephrins and ephrin tyrosine kinase receptors (Ephs) (reviewed in ref. 28). It plays an important role in axon guidance and neuronal cell migration, but also in other cellular migration events. There are six different A-ephrins and three different B-ephrins. The 10 different EphA receptors (EphA1–A10) are promiscuously activated by the six different A-ephrins, while the six different EphB receptors (EphB1–B6) are activated by the three different B-ephrins (B1–B3). A-ephrins are GPI-anchored, while B-ephrins are transmembrane.[28]

Unlike other receptor tyrosine kinases, which require receptor dimerization, Eph signaling requires preclustering of ephrins and Ephs into oligomers. This preclustering occurs at the cell–cell contact region, more specifically in membrane micro-domains or rafts, underscoring the contact-dependence of the signaling. Ligand clustering is followed by Eph clustering in assemblies consisting of hundreds of Eph receptors. The role of the initial ligand clustering is probably to increase the local concentration of the Eph receptors on the cell surface, necessary for efficient downstream signaling. In addition, different subtypes of Eph receptors can cluster with each other. Eph clusters can also interact with EGF, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), chemokine, and N-methyl-D-aspartate (NMDA), resulting in cross-talks and alterations of the downstream signaling.[28] The membrane metalloprotease A disintegrin and metalloproteinase 10 (ADAM10),[52] anchored in the Eph-bearing cell, also associates with the ephrin/Eph cluster. This causes a conformational change, bringing the ADAM catalytic domain in a favorable position for cleavage of the ephrin extracellular domain, in this way terminating Eph signaling and resulting in de-adhesion.[28]

Other peculiarities of ephrin/Eph complexes worth mentioning are that (1) ligand-independent signaling can occur at elevated receptor concentration, as is seen in tumor cells, (2) binding of ephrins not only activates its receptor, but also generates a reverse signal into the ephrin-bearing cell, and (3) ephrins and Eph can be expressed and function on the same cell, resulting in what is called "cis-inhibitory interactions."[28] Cis-signaling has also been observed for delta and Notch,[29] and for SEMA6 and plexin A receptors.[30] Of peculiar interest is that, whereas ephrin forward signaling causes axon repulsion, reverse signaling by Eph results in axon attraction. This occurs by interaction of ephrin-A with the receptor rearranged during transfection (Ret) in the ephrin-bearing cell.[53] In the presence of the Ret ligand, glial cell-derived neurotrophic factor (GDNF), and its co-receptor, GDNF family receptor α1 (GFRα1), axon attraction is potentiated.[53] In contrast, interaction between ephrin-A and the p75 neurotrophin receptor causes reverse signaling from Eph to ephrin-A that results in axon repulsion.[53]

Notch ligands. The Notch signaling pathway is a highly conserved juxtacrine cell communication system, involved in cell-fate specification, formation of growth-organizing boundaries, stem cell maintenance, proliferation, apoptosis, and migration.[1] *Drosophila* Notch ligands are delta and serrate; mammalian canonical Notch ligands are Jagged 1 (Jag1), Jag2, delta-like 1 (Dll1), and Dll4.[54] They signal through four different single-pass transmembrane receptors (Notch1–4). Both receptors and ligands have extracellular domains with many EGF-like repeats, namely 29–36 repeats in Notch receptors, and 6–16 of these repeats in the ligands. The EGF-like repeats, together with the N-terminal domain of the ligands (DSL domain), are involved in receptor–ligand binding.[54]

A notable characteristic is that Notch requires three steps of regulated proteolysis to become operational (reviewed in ref. 29 and 55). A first proteolytic step by furin cleaves the receptor precursor in two parts: an extracellular binding domain and a transmembrane/intracellular domain, which remains non-covalently bound to the extracellular domain; in this way, Notch behaves as a heterodimer. The second proteolysis occurs upon ligand binding, which triggers the cleavage of the Notch extracellular domain by ADAM10 or ADAM17 metalloprotease. This step is followed by intramembrane proteolysis by γ-secretase, which releases the intracellular Notch domain (ICD) that then translocates to the nucleus. There, ICD forms a transcription complex with a DNA-binding factor and co-activators, resulting in transcription of target genes. In contrast to other signaling systems, canonical Notch activation does not produce second messengers; hence, it is not amplified and, because of the short ICD half-life, it is of short duration.[29]

Another distinctive feature is the negative regulatory region in Notch, located C-terminal to the EGF-like domains. In the absence of ligand, the folding of that region protects Notch from proteolysis by ADAMs, and thus prevents signaling. Furthermore, Notch ligands require endocytosis into the signaling cell, a process involving ubiquitin-, clathrin-, dynamin-, and epsin-dependent steps, and they must be recycled back to the cell surface prior to engagement with Notch (reviewed in ref. 54 and 55). It has been proposed that endocytosis of the ligand into the signal-sending cell pulls the Notch extracellular domain on the signal-receiving cell toward the signal-sending cell, resulting in a conformational change that permits ADAM10 and ADAM17 proteases to cleave Notch and to ultimately activate the receptor.[55]

A final striking characteristic of Notch signaling is that it can be modulated in a time- and space-dependent manner by accessory proteins (reviewed in ref. 54). Some are type I transmembrane proteins, such as delta-like homolog 1 (Dlk-1) and Dlk-2, low density lipoprotein receptor-related protein 1 (Lrp1), and delta/Notch-like EGF-related receptor (DNER). Other accessory proteins are GPI-anchored such as contactin 1 and 6 and several are soluble, such as thrombospondin-2 (Tsp2) and EGF-like domain 7 (EGFL7). DNER

expression in the signal-sending cell binds Notch and stimulates Notch signaling in the signal-receiving cell. Tsp2 binds to Notch3 and enhances Notch signaling, Lrp1 being essential for the latter effect. Interestingly, EGFL, Tsp2, and Lrp1 bind to both Notch and canonical Notch ligands. Although transmembrane ligands are the dominant biologically active canonical Notch ligands, the Jag1 extracellular domain can be shed by β-secretase 1 (BACE 1),[56] and can display specific biological activities as a soluble molecule, such as inducing FGF receptor-dependent cell transformation in NIH3T3 fibroblasts[57] and keratinocyte differentiation.[58]

Differential roles of juxtacrine and auto/paracrine signaling. Although there are many examples of the membrane-anchored form of a growth factor being the prototype active molecule (eg ephrins, canonical Notch ligands, and class 4, 5, 6, and 7 semaphorins),[30] it has become clear that the extracellular domain can be shed by transmembrane metalloproteinases to become a soluble paracrine or autocrine substance, with both the membrane-bound and the soluble form being biologically active.[59] Examples are Kit ligand,[27] colony-stimulating factor 1 (CSF-1),[60] TNFα,[15] and EGF family members among others.[2] Importantly, qualitative differences have been observed between the biological effects of the two forms. Experiments with MDCK epithelial cells, transfected with either a non-cleavable membrane-anchored heparin-binding EGF (HB-EGF) mutant or a secreted HB-EGF, have demonstrated that cells exposed to soluble HB-EGF display decreased cell–cell and cell–ECM interactions and increased migration, while cells expressing the membrane-anchored HB-EGF display enhanced interactions and decreased migration.[61] Cells expressing the non-cleavable form of HB-EGF also show increased survival from anoikis, a form of programmed cell death induced when anchorage-dependent cells detach from the surrounding ECM.[62] Exposure to the membrane-anchored HB-EGF resulted in more cell aggregation and maintenance of epithelial characteristics even following prolonged detachment from the substratum.[62] Evidence for opposite effects of membrane-anchored and soluble HB-EGF on mitosis and apoptosis was also found in human luteinizing granulosa cells.[63] A growth inhibitory and pro-apoptotic effect of membrane-anchored HB-EGF was seen in EGFR-expressing DER cells (a bone marrow-derived cell line) co-cultured on a monolayer of Vero-H cells (African green monkey kidney cell line) over-expressing membrane-anchored HB-EGF; in contrast, in DER cells cultured alone, soluble HB-EGF stimulated growth.[64] Soluble Dlk1 was found to inhibit adipocyte differentiation in vitro, while the membrane-anchored protein promoted differentiation.[65] In contrast, both membrane-bound Kit ligand and the soluble form have the same effect on hematopoiesis.[66] Remarkable differences in action have also been reported for the TNF ligand superfamily.[67] The transmembrane form of TNF is superior to soluble TNF in activating TNFR2, while soluble TNFα activates TNFR1.[68] Transmembrane TNF and transmembrane Fas ligand induce apoptosis in cancer cells, while their soluble ligands are weakly cytotoxic or block apoptosis mediated by the corresponding transmembrane ligands.[69] In addition, membrane-bound Fas ligand induces inflammation, while soluble Fas ligand suppresses it.[69]

Since membrane-anchored growth factors can have their own specific activities, shedding must be tightly regulated.[70,71] Various signaling molecules, such as cannabinoids, IL8, TGFβ, TNFα, IL1β and gastrin-releasing peptide, have been found to induce shedding or to alter the shedding rate (reviewed in ref. 11).

Juxtacrine signaling after shedding of transmembrane growth factors. Shedding may not abolish juxtacrine signaling by transmembrane growth factors. Experiments with ADAM metalloproteinase inhibitors have suggested that the juxtacrine mode of signaling of membrane-anchored growth factors can be associated with shedding. Blocking TGFα shedding with an ADAM inhibitor was found to inhibit growth and migration in several EGFR-dependent cell lines[72] and to retard wound re-epithelialization.[73] In co-cultures of CHO cells (Chinese hamster ovary cells) transfected with TGFα and EGFR-expressing A431 cells (epidermoid carcinoma cells), treatment with a shedding inhibitor abolished EGFR activation, although adhesive juxtaposition of TGFα-expressing cells with EGFR-expressing cells did still occur.[74] Thus, it is possible that the signaling mode by membrane-anchored EGF ligands is juxtacrine, but that shedding is indispensable for EGFR activation. It is conceivable that the adhesion architecture stringently keeps the shed ligands near the receptors. This may be achieved by components in the ECM (see next section).

ECM-dependent Juxtacrine Signaling

Contact-dependent signaling can also occur through molecules released from the signaling cell if the area of distribution remains restricted to the immediate vicinity of the receptor on the receiving cell. This is, for example, the case for neurotransmitters in neuronal synapses, but several other secreted molecules may signal in a local contact-dependent manner by associating with ECM components, transmembrane accessory proteins, and adhesion molecules.

Presentation of secreted ligands by HSPGs and other ECM components. (Fig. 2) various secreted or shed ligands can remain tightly associated with the ECM, thereby increasing local concentration and creating positional information.[75] A sequestered growth factor can be released upon proteolytic processing of the ECM, such as during injury and inflammation.[76] A secreted growth factor can also be presented to the receptor in association with the ECM. ECM association may be mandatory for receptor activation or may modulate the activation in a positive or negative way.[76] Obviously, such a signaling mode requires close contact between the receiving cell and the ECM-associated signal and can therefore be considered as a juxtacrine signal.

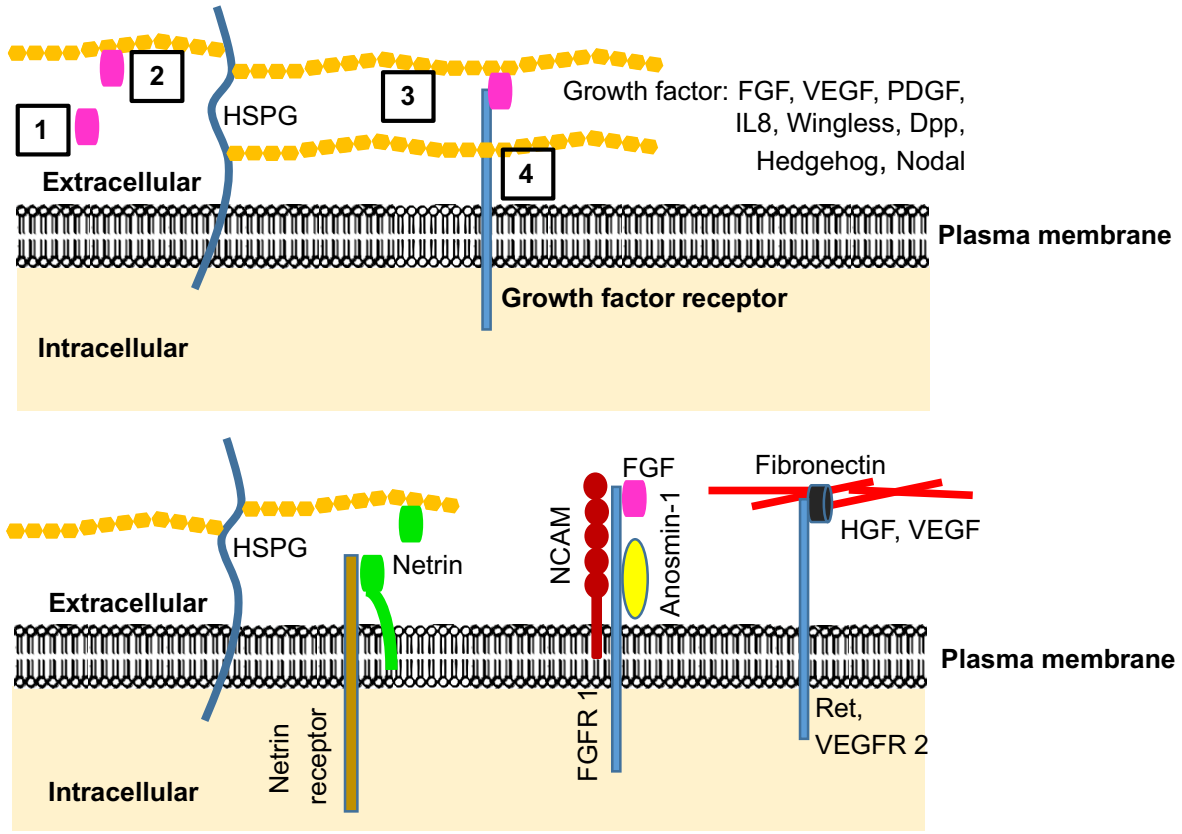

Figure 2. Schematic depiction of the association of various growth factors and/or their receptors with various ECM components eg HSPGs, netrins, NCAM, fibronectin, and anosmin 1. Association leads to enhanced or attenuated signal transduction. A soluble growth factor can remain soluble (**1**), captured by HSPGs (**2**), or presented to the receptor in an HSPG-associated form (**3**). For FGF signal transduction binding of both FGF and FGFR to the HSPG is mandatory (**4**).

The molecules involved in this mode of signaling are often HSPGs. HSPGs include perlecan, agrin, collagen type XI, syndecans, and glypicans. The first three are secreted into the ECM, while syndecans and glypicans are transmembrane and GPI-anchored, respectively. They can be shed from the cell surface by proteases and phospholipases, respectively. Signals presented in association with HSPGs during embryonic development include Wingless, Hedgehog (Hh), Decapentaplegic (Dpp), and Nodal.[4] Other examples include FGFs and VEGFs.[6,77] FGFs activate their receptors (FGFRs) with the obligatory help of HSPGs; the HS moiety has to bind to both FGF and the FGFR. HS chains of perlecan favor FGF/FGFR interaction, while chondroitin sulfate chains in perlecan act as a negative regulator by sequestering the FGFs from their cognate receptors.[78] HSPGs are also involved in platelet-derived growth factor (PDGF) signaling, but they differ from those activating FGF signaling.[76] Activation of the IL8 receptors CXCR1 and CXCR2 on granulocytes requires binding of IL8 to sulfated proteoglycans of the cell surface and the ECM, and IL8 acts in concert with selectins and integrins.[79]

Other examples of ECM components associating with growth factors are fibronectin and vitronectin. They bind hepatocyte growth factor (HGF) and form complexes with the HGF receptor Met and with integrins, which either positively or negatively affects HGF action.[75] The fibronectin III (FnIII) domains in fibronectin bind to VEGF, which potentiates VEGF signaling through VEGFR2.[75]

ECM components may also associate with growth factor receptors. FnIII domains of the ECM-associated protein anosmin-1 (the product of the *KAL1* gene, responsible for the X-linked form of Kallmann syndrome) bind to the FGFR1 ectodomain and function as a co-ligand for the FGFR1 signaling complex, enhancing the activity.[75] FnIII domains of neural cell adhesion molecule bind directly to FGFR1 resulting in ligand-independent FGFR activation.[75] ECM components can also regulate the expression level of a growth factor or its receptor.[76]

Other matrix components important in signaling are netrins (Fig. 2). The netrin family includes laminin-like secreted proteins and GPI-anchored proteins. Netrins were originally discovered as axon guidance and repulsion cues during neural development, but later found to also have general developmental roles in cell migration, cell–cell interactions, and cell–matrix adhesion (reviewed in ref. 80). Canonical netrin receptors are type I transmembrane proteins belonging

to the immunoglobulin (Ig) superfamily. Secreted netrins tightly associate with HS through the C-terminal domain.[80] Netrin 4, but not netrin 1 or 3, can be incorporated into basement membranes of various tissues through interaction with domain VI of laminin. In this way, netrin 4 influences organogenesis by signaling to cells from the basement membrane or from HSPG-stored sites.[80]

Juxtacrine signaling by phospholipids. Juxtacrine signaling can also be the mode of action of non-protein molecules, such as the phospholipids sphingosine-1-phosphate (S1P) and platelet-activating factor (PAF). S1P is synthesized intracellularly by sphingosine kinase (SphK), upon activation of that enzyme by different cytokines and other inflammatory molecules in various immune cells. It plays a crucial role in establishing cell–cell and cell–matrix adhesion (reviewed in ref. 81). S1P acts inside the cell but also extracellularly via G-protein coupled receptors, where it is believed to act locally in a cell contact-dependent manner.[82] S1P can be exported from cells via different transporters,[82] but can also be synthesized extracellularly by SphK that itself can be translocated to the plasma membrane.[83]

PAF is a phospholipid involved in platelet aggregation and degranulation, inflammation, anaphylaxis, and chemotaxis of leukocytes. It can be retained on the surface of cells and signal to its G-protein coupled receptor on juxtaposed cells. The action of PAF requires cooperation of co-expressed P-selectin.[84] PAF signals while P-selectin tethers the receiving cell, creating the juxtacrine context.

Juxtacrine signaling by integrins. (Fig. 3) in normal cells, growth factor receptors often signal inefficiently in the absence of cell adhesion, and loss of adhesion can cause growth arrest and anoikis (detachment-induced apoptosis).[75] Integrins

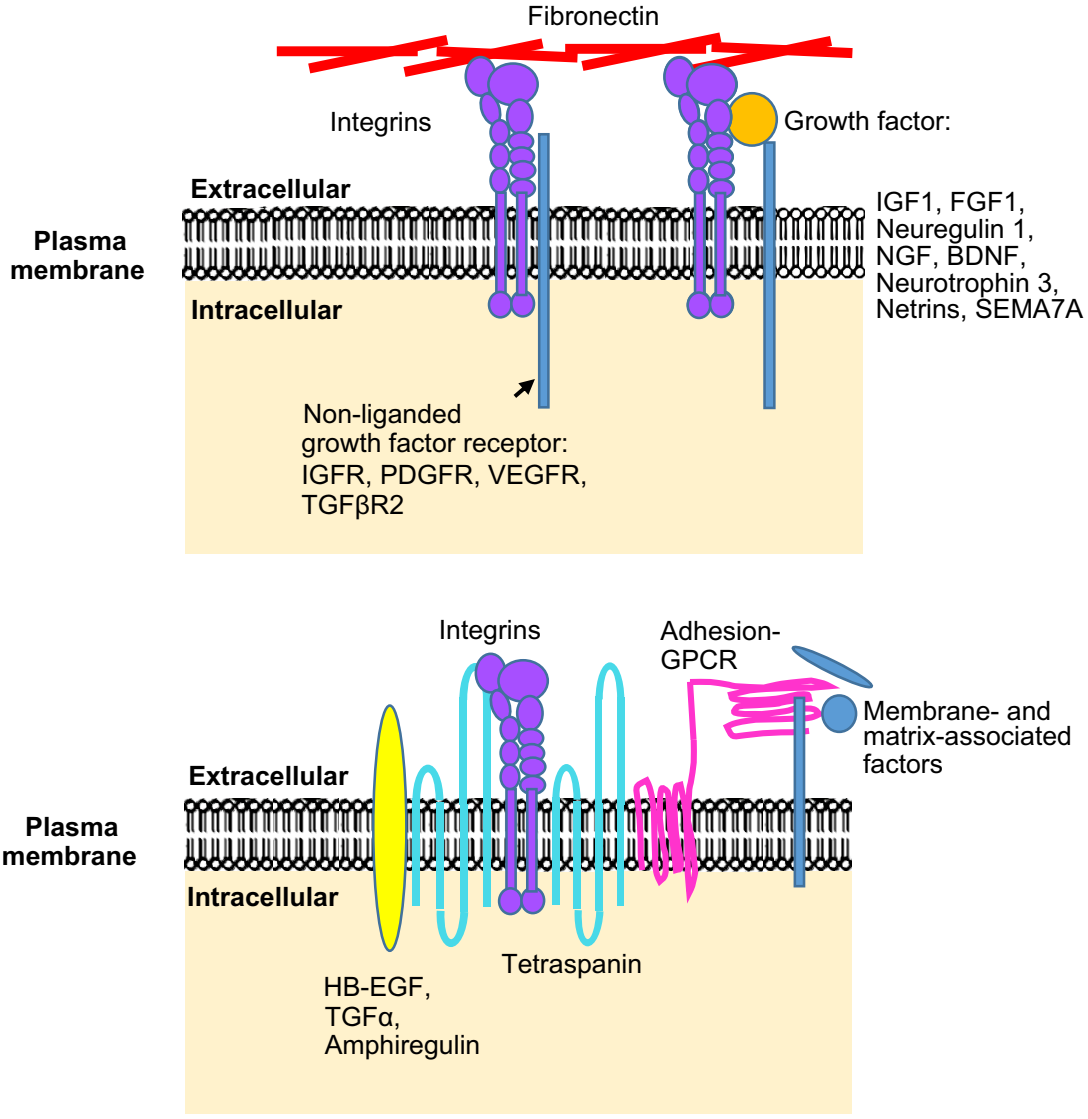

Figure 3. Schematic depiction of the association of various growth factors or their receptor (adhesion GPCR) with tetraspanins and integrins in plasma membrane microdomains, leading to enhanced signal transduction. The same growth factor can also augment integrin expression.

are among the best studied adhesion molecules. They have an essential role in attaching cells to the ECM by binding to ECM ligands, such as fibronectin, vitronectin, collagen, and laminin. There are 18 integrin α-subunits and 8 β-subunits, which can combine into 24 different heterodimers.[85]

Integrins affect signaling by modulating the cell signaling pathways. They can associate with and activate growth factor receptors in a growth-factor independent manner.[77,85] For example, αVβ3 integrin activates insulin-like growth factor (IGF) receptor 1 (IGFR1), platelet-derived growth factor receptor (PDGFR), and VEGFR2.[86] Often the signaling is bidirectional. For example, HGF increases the expression of a subset of integrins, including α2β1 integrin,[87] whereas binding of α5β1 integrin to fibronectin results in ligand-independent activation of the HGF receptor Met.[88] Upon exposure of lung fibroblasts to TGFβ1, the TGFβR2 clusters with αVβ3 integrin, dramatically enhancing proliferation induced by TGFβ1, while TGFβ upregulates αVβ3 integrin expression.[89] Interestingly, different cell types express different integrins, which creates different cellular contexts and therefore will determine whether a growth factor will be more effective or less.[75]

Specific integrins also bind to growth factors.[77] For example, αVβ3 integrin can bind to FGF1, IGF1, or neuregulin 1 and promote signaling through the corresponding receptors. Nerve growth factor (NGF), brain-derived neurotrophic factor, and neurotrophin 3 bind to α9β1 integrin, stimulating cell proliferation and migration. NGF binding to α9β1 also activates integrin-dependent signaling pathways.[77] Finally, a growth factor may bind to both integrin and its cognate receptor and induce a complex between the growth factor, its receptor, and the integrin.[77] Sometimes, the integrin-binding motif RGD is present in the growth factor's amino acid sequence, such as in the propeptide of latent TGFβ, VEGF-A, and angiopoietins. Integrin αVβ8 plays a major role in the activation of latent TGFβ, stored in the ECM.[77] Integrins α6β4 and α3β1 also bind netrin-1, thereby altering epithelial cell adhesion and migration.[80] Integrin α1β1 also serves as a signaling receptor for semaphorin 7A.[77]

Juxtacrine signaling by tetraspanins. (Fig. 3) tetraspanins, such as CD9, CD63, CD81, and CD82, are transmembrane proteins with four transmembrane domains that are found in nearly all cell types and have a role in cell adhesion, motility, cell proliferation, and ECM degradation and rearrangement.[90] Tetraspanins associate with other tetraspanins to form "tetraspanin-enriched microdomains" and with integrins, clustering them in these microdomains on the cell surface, thereby functioning as molecular architecture for cell adhesion and efficient signal transduction. Association of the tetraspanin CD9 with HB-EGF or amphiregulin in renal epithelial cells upregulates the mitogenic activity of the latter and increases proHB-EGF's cytoprotective capacity.[2] CD9 also interacts with β1 integrins, strengthening adhesion at the adherens junctions.[2] In Vero cells expressing HB-EGF and CD9, the HB-EGF–CD9–integrin clusters are found in the cell–cell contact zone in association with vinculin and α-catenin.[12] In MDCK epithelial cells, co-expression of TGFα with CD9 increases and stabilizes the transmembrane form of TGFα at the cell surface with a striking presence at the apical membrane; these cells show increased adhesion in comparison with MDCK cells that express TGFα alone.[91] CD81 has an important role in the molecular organization and dynamics of the immunological synapse.[92] CD9 and CD151 accumulate at the T-cell side of the immunological synapse, thereby relocalizing α4β1 integrin and high-affinity β1 integrins at the cell–cell contact zone.[93] It should also be mentioned here that tetraspanins associate with adhesion-G-protein-coupled receptors (adhesion-GPCRs), a large family of GPCRs with extremely long extracellular N-terminals that contain a wide variety of domains, capable of interacting with many transmembrane and matrix-associated molecules.[94] Thus, taken together, tetraspanins may be important components in the architecture of contact-dependent intercellular signaling.

Juxtacrine Signaling by Receptor-captured Ligands: A Positional Cue

A peculiar mode of juxtacrine signaling by a secreted molecule has been reported for the self-avoidance process between dendrites of the nociceptive PVD neurons in *C. elegans*.[31] Dendrites from a single neuron are highly branched but rarely touch each other. In these dendrites, netrin was shown to be sequestered at the surface of the dendritic branches by the netrin receptor uncoordinated-40 (UNC-40)/deleted in colorectal cancer (DCC), and, upon direct contact with a sister branch, bound netrin was found to interact with another receptor, UNC-5, on the juxtaposed dendrite, initiating a repulsive response.[31] In *Drosophila*, the netrin receptor Frazzled was shown to capture secreted netrin and to present the bound ligand as a guidance cue for recognition by other receptors on nearby neurons.[95] Frazzled also re-localizes netrin along axons, creating positional information for netrin.

Contact-dependent Signaling Via Cytonemes

Cytonemes were originally described by Ramirez-Weber and Kornberg in *Drosophila* as very thin actin-based cellular extensions (~200 nm diameter) that project from the wing imaginal disk cells to the morphogen signaling center, located in a narrow stripe on the anterior side of the anterior/posterior compartment border of the wing disk, where Dpp, the homolog of the vertebrate bone morphogenetic proteins, is expressed.[7] They extend for distances up to 20 times the diameter of a disk cell. Signaling through cytonemes was subsequently demonstrated in *Drosophila* for Dpp from the wing imaginal disk to the air sac primordium (ASP),[96] and for Hedgehog (Hh) in wing disk cells and abdominal epidermis[97] and in the ovary germline stem cell niche.[98]

The ASP is juxtaposed to the basal surface of the wing disk, and its development is dependent on Dpp and FGF, both produced by the disk cells. Cells at the ASP tip develop long cytonemes (≥30 μm in length), most of which project toward the FGF-containing disk cells, and some to Dpp cells. ASP cytonemes take up Dpp from the wing disk and translocate the molecules in motile "puncta" along the ASP cell's cytonemes. ASP cells express the Dpp receptor Thickveins (Tkv) and the FGF receptor Breathless, and segregate each of these receptors to puncta in distinct cytonemes, suggesting cytonemes are ligand specific.[99] Cytoneme tips synapse with wing disk cells, the distance between a cytoneme tip and the target cell being less than 20 nm, comparable with the space between presynaptic and postsynaptic membranes of neuronal synapses.[96]

The *Drosophila* ovary contains a stem cell niche that hosts two to three germline stem cells. Hh is produced in niche support cells (the cap cells), and it is translocated to a neighboring population of niche cells (the escort cells) via cytonemes originating in the cap cells. Hh is delivered to the escort cells, where the Hh pathway is activated.[98] Importantly, under experimental conditions that create low levels of Hh protein within the niche, cap cells emit up to six-fold longer Hh-containing cytonemes toward the signaling-deficient area of the niche, suggesting that the cytoneme communication system is regulated.[98]

In developing limb buds in chick embryos, both Hh-producing and -responding cells can extend cytonemes containing Hh in the form of particles that move along these extensions.[100] They are 200 nm in diameter and up to 150 μm in length and display unique cytoskeletal features. Hh particles travel in both anterograde and retrograde directions along the cytonemes, with a net anterograde movement away from the cell body, at a maximum velocity of 120 nm s^{-1}. Cytonemes of Hh-responding cells display Hh co-receptors in microdomains on the external surface of the cytonemes. Stabilized interactions are formed between cytonemes containing Hh and those containing co-receptors.[100]

Cell–cell interaction through cytonemes or cytoneme-like extensions has also been reported in Notch signaling in *Drosophila*,[54] during B cell activation, between mast cells, and in neutrophils exposed to nitric oxide.[7]

Conclusion

The present overview illustrates how cells can diversify message and response through adhesive cell–cell and cell–matrix interactions and by directed signal translocation. Adhesive cell–cell interaction underlies spatially restricted signaling by membrane-anchored growth factors, while secreted molecules can form local signaling centers through association with ECM components, accessory transmembrane proteins, and adhesion molecules. Adhesive cell–cell and cell–matrix interactions and receptor-captured signals can create contextual and positional information. Signals can be guided to specified targets at a distance via signal-specific cytonemes and delivered through synapse-like structures to signal-receptive cells.

Acknowledgments

The author is grateful to Natalie Denef for critically reading the manuscript.

Author Contributions

Conceived the concepts: CD. Analyzed the data: CD. Wrote the first draft of the manuscript: CD. Made critical revisions: CD. The author reviewed and approved of the final manuscript.

Abbreviations

ADAM, A disintegrin and metalloproteinase; ASP, air sac primordium; BST-1, bone marrow stromal cell antigen 1; CD, cluster of differentiation; CSGP: chondroitin sulfate proteoglycans; Dlk, delta-like homolog; Dpp, decapentaplegic; ECM, extracellular matrix; EGF, epidermal growth factor; Eph, ephrin receptor; FGF, fibroblast growth factor; GPCR, G protein-coupled receptor; GPI, glycosylphosphatidylinositol; HB-EGF, heparin-binding EGF; HGF, hepatocyte growth factor; Hh, Hedgehog; HS, heparan sulfate; HSPG, heparan sulfate proteoglycans; ICD, intracellular Notch domain; Jag, jagged; PAF, platelet-activating factor; NPR, neuropilin; PDGF, platelet-derived growth factor; R, receptor; S1P, sphingosine-1-phosphate; SEMA, semaphorin; TGFα, transforming growth factor-α; TGFβ, transforming growth fact β; TNF: tumor-necrosis factor; VEGF, vascular endothelial growth factor.

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