

Review

Cross-talk between RON receptor tyrosine kinase and other transmembrane receptors

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Summary. RON is a transmembrane receptor tyrosine kinase that mediates biological activities of Macrophage Stimulating Protein (MSP). MSP is a multifunctional factor regulating cell adhesion, motility, growth and survival. MSP binding to RON causes receptor tyrosine phosphorylation leading to up-regulation of RON catalytic activity and subsequent activation of downstream signaling molecules. Recent studies show that RON is spatially and functionally associated with other transmembrane molecules including adhesion receptors integrins and cadherins, and cytokine and growth factor receptors IL-3 β c, EPOR and MET. For example, MSP-induced cell shape change is mediated via RON-activated IL-3 β c receptor. Activation of integrins causes MSP-independent RON phosphorylation, and the integrin/RON collaboration regulates cell survival. Thus, RON can be activated without MSP by ligand stimulation of RON-associated receptors, and MSP-activated RON can cause ligand-independent activation of RON-associated receptors. As a result of the receptor cross-activation RON-specific pathways become a part of a signal transduction network of other receptors, and conversely signaling pathways activated by other receptors can be used by RON. This receptor collaboration extends the spectrum of cellular responses generated by MSP and by putative ligands of RON-associated receptors. However signaling pathways involved in the receptor cross-talk and underlying activation mechanisms remain to be investigated. The purpose of this review is to summarize data and to discuss a role of cross-talk between RON and other transmembrane receptors.

Key words: RON, Transmembrane receptors, Cross-talk

Introduction

RON (Receptuer d'Origine Nantaise) is a transmembrane receptor tyrosine kinase (RTK) belonging to the MET RTK family. The RON gene was cloned from human keratinocytes (Ronsin et al., 1993), and the mouse homologue, known as STK (Stem cell derived Tyrosine Kinase), was cloned from mouse hematopoietic stem cells (Iwama et al., 1994). The RON gene encodes a 190 kDa protein, which is expressed on the cell surface as a disulfide-linked heterodimer comprising 40 kDa α and 150 kDa β chains (Angeloni et al., 2000). Single chain 190 kDa pro-RON has a basic amino acid site KRRRR, intracellular proteolytic cleavage of which converts pro-RON into the mature, two chain heterodimeric receptor (Gaudino et al., 1994). The α chain of RON is located in the extracellular part of the receptor, whereas the β chain has extracellular, transmembrane and cytoplasmic domains (Fig. 1). The extracellular part of RON binds MSP; the cytoplasmic part of RON has an intrinsic tyrosine kinase activity, which is up-regulated by MSP binding to RON (Gaudino et al., 1994).

The ligand for RON is Macrophage Stimulating Protein (MSP) (Wang et al., 1994), also known as Hepatocyte Growth Factor-like protein (HGFL). MSP was discovered as a serum factor that regulates the motility of macrophages (Leonard and Skeel, 1976). Recent investigations have shown that the RON is expressed on various cell types including macrophages (Iwama et al., 1995), osteoclasts (Kurihara et al., 1996), epithelial (Wang et al., 1996a) and hematopoietic cells, such as erythroid and myeloid progenitor cells (Iwama et al., 1996) and bone marrow megakaryocytes (Banu et al., 1996). Interaction of MSP with RON causes receptor tyrosine phosphorylation and activation, a key event in signal transduction from cell surface to cell interior (Leonard and Danilkovitch, 2000).

Signaling pathways activated by MSP/RON interaction mediate cell adhesion, motility, growth and survival (Wang et al., 1996a,b; Danilkovitch et al., 1999, 2000; Danilkovitch and Leonard, 1999; Leonard and Danilkovitch, 2000). Recent investigations have shown

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that RON kinase activity and RON-activated signaling pathways can be regulated through other receptors by MSP-independent mechanisms. RON can be a functional part of other receptor complexes activated by stimuli that do not directly interact with RON, and RON can transduce signals and regulate cellular responses induced by these stimuli. On the other hand, some biological activities induced by MSP/RON interaction are mediated by activation of other receptors.

The cross-talk between RON and other receptors generates additional signal diversity, resulting in a wide spectrum of cellular biological responses that regulate normal cell functional activity as well as steps in oncogenic cell transformation.

RON and integrins

Integrins are cell surface receptors that mediate cell adhesion to extracellular matrix proteins (ECM). Integrins are heterodimers consisting of non-covalently linked transmembrane α and β chains (Hynes, 1992). Integrin/ECM-mediated adhesion activates intracellular signaling pathways, which in addition to cell adhesiveness also regulate cell growth and survival (Hanks and Polte, 1997; Kumar, 1998; Giancotti and Ruoslahti, 1999). These signaling pathways include a number of intracellular kinases and phosphatases such as Focal Adhesion kinase (FAK), c-Src, MAPK, phosphatidylinositol-3 kinase (PI-3 kinase) and phosphatase SHP-2 (Cary et al., 1999). Integrins can physically associate and functionally cooperate with other receptors including growth factor RTKs (Juliano, 1996; Miyamoto et al., 1996; Sundberg and Rubin,

1996; Wang et al., 1996; Porter and Hogg, 1998; Soldi et al., 1999).

We have shown by co-immunoprecipitation that RON is physically associated with the β chain of integrins (Danilkovitch et al., 1999). Plating of RON-expressing epithelial cells on ECM causes ligand-independent RON tyrosine phosphorylation and kinase activation (Danilkovitch-Miagkova et al., 2000). These experimental data show that RON can be tyrosine phosphorylated and activated in the absence of its ligand MSP via ECM/integrin-dependent adhesion. ECM-dependent RON activation is a multi-step process that requires both RON and c-Src kinase activities. Because RON is associated with $\beta 1$ integrin (Danilkovitch et al., 1999), ECM-induced integrin aggregation may lead to RON oligomerization and transphosphorylation by RON kinase. This autophosphorylated RON becomes a target for c-Src activated by ECM/integrin dependent adhesion, which results in the phosphorylation of additional tyrosines on RON (Danilkovitch-Miagkova et al., 2000).

When MSP is added to cells plated on ECM, the resulting kinase activation and phosphorylation of RON is greater than the levels observed for cells on ECM alone or for cells stimulated by MSP in suspension.

In light of these results and data from the literature, we can identify candidate tyrosines on RON that are phosphorylated in response to MSP or to plating on ECM. Because phosphorylation of tyrosines Y1238 and Y1239 is essential for up-regulation of RON catalytic activity (Gaudino et al., 1994; Longati et al., 1994; Iwama et al., 1996), we suggest that these tyrosines are phosphorylated by either the ECM-dependent mechanism or by MSP. Additional evidence for their role in response to plating is the fact that ECM does not trigger tyrosine phosphorylation of kinase dead RON (Danilkovitch-Miagkova et al., 2000) indicating that ECM induces autophosphorylation of Y1238/Y1239 in RON.

Once activated, RON kinase can phosphorylate tyrosines Y1353 and Y1360 representing the RON C-terminal multifunctional docking site for downstream signaling molecules (Ponzetto et al., 1994; Iwama et al., 1996). Thus, either MSP or ECM can trigger Y1238/Y1239 and Y1353/Y1360 autophosphorylation in RON. In addition to these common target tyrosines, there are additional phosphorylation sites that are specific for one or the other stimulus. An MSP-specific RON tyrosine is postulated because of the phosphorylation increment observed when MSP is added to cells plated on ECM. On the other hand, ECM-specific RON tyrosine phosphorylation occurs because auto-phosphorylated RON from plated cells (but not from MSP-stimulated cells in suspension) is a target for c-Src kinase, which is activated via a FAK pathway in response to ECM/integrin interaction (Hanks and Polte, 1997; Giancotti and Ruoslahti, 1999).

What are c-Src phosphorylation sites in RON? Although those sites have been not mapped, we can suggest potential c-Src phosphorylation sites in RON on

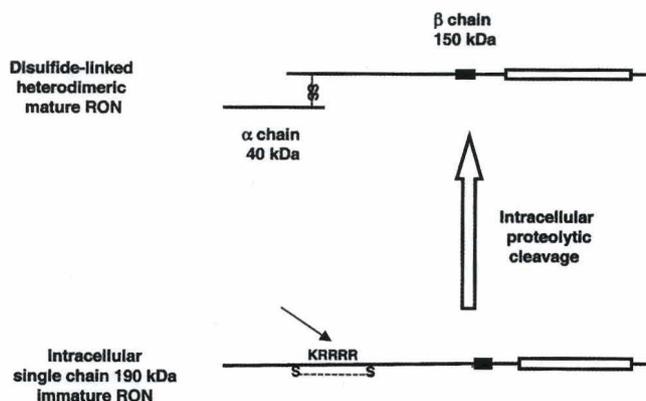


Fig. 1. Schematic representation of immature (intracellular) and mature (expressed on cell membrane) RON. RON is synthesized as a 190 kDa single chain protein. It has a basic amino acid site KRRRR (amino acid residues 305-309, indicated by the closed arrow), proteolytic cleavage of which inside the endoplasmic reticulum converts single chain immature RON into $\alpha\beta$ chain heterodimeric mature RON. The black box represents the RON transmembrane domain (amino acid residues 958-982). The open box represents the kinase domain (amino acid residues 1089-1352). The S-S symbol shows disulfide bridges between α and β chains of RON. The number and the location of these bonds are still unknown.

RON cross-talk with other receptors

the basis of tyrosine motifs of the c-Src-dependent phosphorylation sites that have been identified in the EGFR (Gotoh et al., 1992; Sato et al., 1995; Stover et al., 1995; Biscardi et al., 1999; Tice et al., 1999). The three potential sites (Y1198, Y1288, Y1317) and their motifs are listed in Table 1.

How can RON be involved in ECM/integrin-induced signal transduction? Just as there are common and unique RON phosphorylation sites depending on the stimulus, the same applies to resultant signal transduction. Thus, either MSP or ECM plating should generate docking sites on RON for signaling proteins with SH2 or PTB domains. Likewise, either stimulus increases RON kinase catalytic activity, which results in phosphorylation of RON substrates involved in signal transduction. On the other hand, the data that c-Src can phosphorylate RON from cells stimulated by ECM, but not by MSP (Danilkovitch-Miagkova et al., 2000), can be the basis for a signaling pathway that is unique to the ECM stimulus.

A possible signal in this pathway is Stat3. Analysis of tyrosine motifs in RON reveals that Y1101 has a motif Y-I-D-Q (Table 1), which matches a consensus motif Y-X-X-Q for Stat3 transcriptional factor binding (Coffer and Kruijer, 1995; Stahl et al., 1995). MSP stimulation does not cause Stat3 interaction with the RON in epithelial cells, but Stat3 is co-immunoprecipitated with ECM-activated RON (unpublished data). It suggests the hypothesis that

Y1101 might represent one of the tyrosines that is responsible for specificity of ECM-initiated RON signaling.

It is also possible for signaling to depend on the combination of MSP and ECM stimuli. For example, we have shown that MSP promotes survival of epithelial cells in serum-free medium via two independent pathways, one involving PI3-kinase and the other involving MAPK. If the PI3-kinase pathway is blocked, MSP prevents apoptosis of adherent cells, but not cells in suspension. Although MSP activates MAPK of either suspended or adherent cells, translocation of MAPK from cytoplasm to nucleus occurs only in the adherent cells (Danilkovitch et al., 2000). Thus, the combination of ECM and MSP may activate pathways that are activated by neither MSP nor by adhesion alone.

Additional signaling diversity might be generated by spatial availability of signaling molecules and RON substrates due to differences in MSP or ECM-induced RON receptor aggregation. For example, it has been shown that ECM can increase association between tyrosine specific phosphatase SHP-2 and β PDGFR due to enhancement in the availability of SHP-2 to the PDGFR (DeMali et al., 1999). Another example is that cross-linking of integrins by ECM not only activates the EGFR, but also results in the spatial juxtaposition of Shc with activated EGFR (Moro et al., 1998; Kuwada and Li, 2000). ECM-induced integrin aggregation can also make available molecules including SHP-2 for association

Table 1. Functional role of tyrosine residues in RON cytoplasmic domain.

N	TYROSINE POSITION IN RON	SEQUENCE FROM -1 TO +3	FUNCTIONAL ROLE
1.	Y1013	L-Y-S-G-S*	Unknown
2.	Y1018	D-Y-R-S-G	Unknown
3.	Y1097	V-Y-H-G-E	Unknown
4.	Y1101	E-Y-I-D-Q	Potential Stat3 binding site, the sequence matches Y-X-X-Q consensus motif (Stahl et al., 1995)
5.	Y1163	P-Y-M-C-H	Unknown
6.	Y1198	E-Y-L-A-E	Potential c-Src phosphorylation site, closest homology to Y845 (E-Y-H-A-G) on the EGFR and Y416 (E-Y-T-A-R) in c-Src (Sto et al., 1995; Biscardi et al., 1999; Kmiecik and Shalloway, 1987; Piwnica-Worms et al., 1987)
7.	Y1238	E-Y-Y-S-V	Autophosphorylation site, autophosphorylated in response to MSP and ECM, can be phosphorylated by activated MET (Gaudino et al., 1994; Longati et al., 1994; Iwama et al., 1996; Follenzi et al., 2000)
8.	Y1239	Y-Y-S-V-Q	Autophosphorylation site, autophosphorylated in response to MSP and ECM, can be phosphorylated by activated MET (Gaudino et al., 1994; Longati et al., 1994; Iwama et al., 1996; Follenzi et al., 2000)
9.	Y1262	T-Y-R-F-T	Unknown
10.	Y1288	P-Y-R-H-I	Potential c-Src phosphorylation site, closest homology to Y891 (P-Y-D-G-I) in the EGFR (Stover et al., 1995)
11.	Y1311	E-Y-C-P-D	Unknown
12.	Y1317	L-Y-Q-V-M	Potential c-Src phosphorylation site, closest homology to Y920 (V-Y-M-I-M) in the EGFR (Stover et al., 1995); it mediates oncogenic properties of M1254T RON mutant (Santoro et al., 2000)
13.	Y1353	H-Y-V-Q-L	Autophosphorylation site, phosphorylation can be induced by MSP, ECM and HGF, a multifunctional docking site (Gaudino et al., 1994; Ponzetto et al., 1994; Iwama et al., 1996; Follenzi et al., 2000)
14.	Y1360	T-Y-H-N-L	Autophosphorylation site, phosphorylation can be induced by MSP, ECM and HGF, a multifunctional docking site (Gaudino et al., 1994; Ponzetto et al., 1994; Iwama et al., 1996; Follenzi et al., 2000)

*: one letter code

with RON. SHP-2 has been found in association with RON in adherent cells stimulated by MSP (unpublished data). It is not known whether the RON/SHP-2 association is direct, but there are several tyrosines in RON, including the C-terminal multifunctional docking site, that match the consensus motif Y-X-X-L/V/I representing a potential binding site for SHP-2 (Eck et al., 1996).

RON and MET receptor

RON and MET belong to the same RTK family and share many common structural features: i) mature receptors are disulfide-linked heterodimers; ii) two tyrosine residues in the kinase domain activation loop of the β chain upregulate the receptor kinase activity; iii) two C-terminal tyrosine residues represent a multifunctional docking site that mediates interactions with multiple SH2-domain containing signaling molecules. Interaction of MSP and HGF with their receptors is believed to induce receptor homodimerization, which causes receptor transphosphorylation and activation (Ullrich and Schlessinger, 1990; Heldin, 1995).

A recent investigation shows that activated MET can transphosphorylate RON, and vice versa. Either HGF stimulation of cells co-expressing wild type MET and kinase inactive RON or MSP stimulation of cells co-expressing wild type RON and kinase inactive MET caused tyrosine phosphorylation of both MET and RON (Follenzi et al., 2000). Thus, tyrosine phosphorylation of the inactive receptor occurred when cells were stimulated with the ligand for the active receptor. Transphosphorylation occurred in MET or RON even with receptor mutants lacking the C-terminal docking sites for signal transducing molecules. This suggests that receptor phosphorylation by the non-corresponding ligand is not mediated by downstream signaling molecules. Mapping of transphosphorylated tyrosines showed that catalytic site tyrosines (Y1234/Y1235 in Met and Y1238/Y1239 in RON) and the C-terminal docking site tyrosines (Y1349/Y1356 in MET and Y1353/Y1360 in RON) are the major transphosphorylation sites (Follenzi et al., 2000).

We have observed tyrosine phosphorylation of kinase-dead RON in RON-transfected MDCK cells expressing endogenous MET receptor. In these cells transphosphorylation of kinase-dead RON occurred when MET was activated by cell plating on an ECM-coated surface (unpublished data). This shows that in addition to HGF other stimuli causing MET activation can induce ligand-independent RON phosphorylation.

Homo- and heterodimers between RON and MET have been detected on the cell surface in the absence of ligand stimulation (Follenzi et al., 2000). This indicates that the receptors are co-localized, and because of spatial proximity one receptor may transphosphorylate another one after activation by the appropriate ligand. Heterodimers between RON or MET and receptors in

different families (ErbB1, ErbB2 or TrkA) have been not detected. It correlated with the absence of transphosphorylation between RON or MET and these other receptors (Follenzi et al., 2000). Thus, the transphosphorylation in this experimental system was confined to the receptor subfamily members.

In contrast to these results, cross-talk between the EGFR and MET has been described. In rat epithelial cells as well in some human tumor cell lines expression of TGF α and its receptor, EGFR cause constitutive ligand-independent tyrosine phosphorylation of MET. This cross-talk between MET and EGFR may have significant implications for altered growth control in tumorigenesis (Jo et al., 2000). Stimulation of cells with EGF may cause ligand-independent tyrosine phosphorylation of PDGFR followed by activation of PI3-kinase (Habib et al., 1998). PI3-kinase is the only kinase found to be associated with the PDGFR in cells exposed to EGF. Therefore, EGFR-mediated tyrosine phosphorylation of the PDGFR could have a number of functional consequences that are dependent on PI3-kinase activity (Habib et al., 1998).

These examples show that heterodimerization between receptor tyrosine kinases from different subfamilies may be more widespread than previously believed. Although heterodimerization between RON and other receptor tyrosine kinases from different families has not been shown, it may occur and have functional import.

What is the possible physiological role of cross-talk between RON and MET? It may lead to more efficient signaling initiated by HGF or MSP. For example, formation of an ErbB2/ErbB3 heterodimer results in more efficient signaling, because ErbB2 itself induces weak activation of PI3-kinase, whereas co-expression with ErbB3 leads to strong activation of PI3-kinase (Wallasch et al., 1995). A similar effect on PI3-kinase activation has been observed in cells expressing the EGFR/PDGFR heterodimer (Habib et al., 1998).

Expression of various members of the EGFR family and cross-talk between the members of the EGFR family plays a leading role in tumor progression (Kim and Muller, 1999). RON and MET are overexpressed in tumor cells (Bardelli et al., 1997; Maggiora et al., 1998; Willett et al., 1998; Wallenius et al., 2000), and cross-talk between RON and MET could lead to sustained signaling that might be critical for tumorigenesis.

RON and interleukin-3 receptor (IL-3R)

RON is expressed on various subsets of hematopoietic cells. Its ligand, MSP, is a multifunctional factor for hematopoietic cells. It may promote or inhibit proliferation or induce cell death (Iwama et al., 1996), or cause megakaryocyte cell differentiation (Broxmeyer et al., 1996). Recently, it has been found that MSP/RON interaction causes morphological changes in an IL-3-dependent cell line (Mera et al., 1999). RON is associated with the IL-3 receptor β c chain in these cells.

Cell stimulation either by MSP or IL-3 induces a similar pattern of protein tyrosine phosphorylation, and causes morphological changes in cells. It appears that MSP-activated RON can directly phosphorylate the IL-3 receptor β chain, followed by association of tyrosine phosphorylated IL-3 β c with intracellular pp90 protein. This pp90 protein seems to be involved in both IL-3 and MSP-induced cell shape changes (Mera et al., 1999). IL3, IL-5 and GM-CSF belong to the same cytokine family, and their receptors have a shared receptor subunit named the common β chain (β c) (de Groot et al., 1998). It is possible that RON will cause tyrosine phosphorylation of β c chain of IL-5 and GM-CSF to transduce signals responsible for MSP-induced cell shape changes, if RON and these receptors are both on the cell membrane. Thus, in addition to IL-3R, IL-5 and GM-CSF receptors are potential RON partners in MSP-activated signaling.

RON and the erythropoietin receptor (EPOR)

Erythropoietin (EPO) is the ligand for the EPOR, a transmembrane protein that is essential for the development and differentiation of committed erythroid progenitor cells (Wojchowski et al., 1999). The interaction between EPO and its receptor initiates signal transduction pathways leading to cell proliferation and consequent increase in red cell mass (Wojchowski et al., 1999). The EPOR plays an important role in the development of hematopoietic disorders, including erythroleukemia (Michiels, 1993; McMullin and Percy, 1999).

Erythroleukemia is a malignant disease, characterized by large numbers of dyserythropoietic erythroblasts in the peripheral blood and bone marrow, splenomegaly and hepatomegaly due to extramedullary dysplastic hematopoiesis, and clinical manifestations of severe anemia (Michiels, 1993; Mazzella et al., 2000).

There is an experimental model in which infection of mice with Friend virus causes erythroleukemia. A number of host genes affect susceptibility to Friend disease (Kumar et al., 1978; Shibuya and Mak, 1982; Best et al., 1996). Recently it has been discovered that the FV2 (Friend virus susceptibility 2) locus, which mediates susceptibility to Friend virus-induced erythroleukemia in mice (Lilly, 1970), encodes a truncated form of RON (Persons et al., 1999). This truncated form of RON might be involved in erythroleukemia development or progression.

In cells that were co-transfected with RON, EPOR and p55 Friend virus protein cDNAs, these proteins were co-immunoprecipitated as a complex (Herley et al., 1999). The association of EPOR with either truncated or full sized RON has been identified. Co-expression of RON and EPOR resulted in EPOR tyrosine phosphorylation. These results suggest that RON is a part of EPOR complex. Functional association between EPOR and IL-3R, or EPOR and c-Kit receptor has been shown previously (Wojchowski et al., 1999). For

example, cell stimulation by Stem Cell Factor (SCF), the c-Kit receptor ligand, induces EPO-independent EPOR tyrosine phosphorylation, and this cross-talk between c-Kit and EPOR is important for erythroid cell proliferation (Wu et al., 1995). Thus, we can speculate that MSP-stimulated RON might cause EPOR tyrosine phosphorylation and signaling, or EPO-activated EPOR through associated kinases (for example, Jak2 or c-Src-related kinases) might cause RON phosphorylation and activation. RON/EPOR cross-talk might be crucial for regulation of normal erythropoiesis as well as in erythroleukemia development.

RON and cadherins/catenins

Activating mutations in growth factor RTKs may result in oncogenic cell transformation and tumor development. A number of human neoplastic syndromes are associated with activating point mutations in a highly conserved region of the tyrosine domains of Kit, Ret and Met receptors (Nagata et al., 1995; Santoro et al., 1995; Tsujimura, 1996; Jeffers et al., 1997; Schmidt et al., 1997). Although RON has not yet been implicated in human neoplasia, experimental mutations in the RON kinase domain (D1232V –"Kit" type and M1254T –"Ret" type mutations) result in a tumorigenic phenotype (Santoro et al., 1998). The investigation of signaling pathways activated by these RON mutants show that β -catenin is constitutively tyrosine phosphorylated in cells expressing mutant RON. The increase in β -catenin was associated with an increase in its target oncogene products, c-myc and cyclins A and D1 (unpublished data). Because increased β -catenin occurs in many human cancers (Korinek et al., 1997; Morin et al., 1997; Rubinfeld et al., 1997; Chan et al., 1999; Morin, 1999; Polakis, 1999; Satoh et al., 2000), accumulated β -catenin initiated by oncogenic RON mutants may be implicated in tumor formation. We also found that both wild type and mutated RON are co-immunoprecipitated with E-cadherin and β -catenin (unpublished data). Although we could not detect tyrosine phosphorylation of cadherin/catenin by RON wild type, the association between RON and cadherins indicates possible receptor cross-talk playing an important role in regulation of cadherin/catenin- and RON-dependent cell functions.

Cadherins are transmembrane receptors that are involved in regulation of cell-cell mediated adhesion (Gumbiner, 1996, 2000). β -catenin (*Drosophila* homolog Armadillo) is a multifunctional intracellular protein discovered as a component of adherent junctions (Ozawa et al., 1989). β -catenin associated with E-cadherin and α -catenin at the plasma membrane regulates cell adhesion, whereas cytoplasmic β -catenin is involved in signal transduction and activation of gene expression (Ben-Ze'ev and Geiger, 1998; Bullions and Levine, 1998; Bienz, 1999). Association with and regulation of cadherin/catenin tyrosine phosphorylation via growth factor RTKs has been shown for MET and EGFR

RON cross-talk with other receptors

Table 2. Effects of ligand-activated receptors on the RON.

RECEPTOR ASSOCIATED WITH RON	LIGAND	EFFECTS OF LIGAND-ACTIVATED RECEPTORS ON RON	BIOLOGICAL EFFECTS
Integrin ($\beta 1$ integrin)	ECM	Tyrosine phosphorylation and activation; increases effects of MSP	Sustained activation of signaling pathways? Protection from apoptosis via the MAPK pathway?
MET	HGF	Tyrosine phosphorylation and activation	Sustained activation of signaling pathways
Cadherin (E-Cadherin)	Cadherin	Unknown	Unknown
IL-3 βc	IL-3	No effect	No effect
EPOR	EPO	Unknown	Unknown
POTENTIAL RON PARTNERS	LIGAND	POTENTIAL EFFECTS OF LIGAND-ACTIVATED RECEPTORS ON RON	POTENTIAL BIOLOGICAL EFFECTS
IL-5 βc	IL-5	No effect	No effect
GM-CSF βc	GM-CSF	No effect	No effect

Table 3. Effects of MSP-activated RON on the RON-associated receptors.

RECEPTOR ASSOCIATED WITH RON	EFFECTS OF MSP-INDUCED RON ACTIVATION ON THE RECEPTOR	BIOLOGICAL EFFECTS
Integrin ($\beta 1$ integrin)	Unknown	Increased integrin-dependent adhesion
MET	Tyrosine phosphorylation and activation	Sustained activation of signaling pathways
Cadherin (E-Cadherin)	Tyrosine phosphorylation of cadherin and β -catenin*	Stabilization of β -catenin*; Increased level of c-myc and cyclin D1 expression. Oncogenic cell transformation pathway?
IL-3 βc	Tyrosine phosphorylation of IL-3 βc	Morphological changes
EPOR	Tyrosine phosphorylation of EPOR	Unknown
POTENTIAL RON PARTNERS	POTENTIAL EFFECTS OF MSP-INDUCED RON ACTIVATION ON THE RECEPTOR	POTENTIAL BIOLOGICAL EFFECTS
IL-5 βc	Tyrosine phosphorylation of IL-5 βc	Morphological changes
GM-CSF βc	Tyrosine phosphorylation of GM-CSF βc	Morphological changes

*: effects of constitutively active mutated RON M1254 or D1232V

(Hoschuetzky et al., 1994; Kanai et al., 1995; Hiscox and Jiang, 1999a,b; Muller et al., 1999). Tyrosine phosphorylation of cadherin and catenin regulates their association/dissociation, playing an important role in regulation of cell adhesion and signaling (Daniel and Reynolds, 1997; Roura et al., 1999). RON wild type may be a component of cadherin receptor complex involved in regulation of cell-mediated adhesion and signaling, whereas oncogenic RON mutants can utilize the cadherin/catenin pathway to cause tumorigenic cell transformation.

Conclusion

RON is spatially and functionally associated with other membrane receptors (data are summarized in Tables 2, 3). Signaling mediated by RON or other receptors is not a direct linear pathway from the cell surface. Receptor cross-talk generates diversity of cellular responses to a single ligand. RON as a part of

multi-receptor complexes is important for regulation of a number of cell functions including adhesion and migration, growth and survival. The data summarized in this review show the biological significance of RON signaling networks, and describe directions for future investigations.

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