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Review Article

Receptor tyrosine kinases: Characterisation, mechanism of action and therapeutic interests for bone cancers

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ABSTRACT

Bone cancers are characterised by the development of tumour cells in bone sites, associated with a dysregulation of their environment. In the last two decades, numerous therapeutic strategies have been developed to target the cancer cells or tumour niche. As the crosstalk between these two entities is tightly controlled by the release of polypeptide mediators activating signalling pathways through several receptor tyrosine kinases (RTKs), RTK inhibitors have been designed. These inhibitors have shown exciting clinical impacts, such as imatinib mesylate, which has become a reference treatment for chronic myeloid leukaemia and gastrointestinal tumours. The present review gives an overview of the main molecular and functional characteristics of RTKs, and focuses on the clinical applications that are envisaged and already assessed for the treatment of bone sarcomas and bone metastases.

1. Introduction

To be able to play their physiological role (intra- and inter-cellular signal transmission and adaptation to changes in the microenvironment), cells must be able to receive, integrate and respond to numerous extracellular messengers. These communications between cells and their environment are made possible through the attachment of molecules considered as messengers to their receptors, identified as effectors (cytokines, growth factors, etc). As proposed by Ehrlich in 1910, “to act, a substance must be fixed." These receptors are essentially located at the cell membrane, although there are also intra-cytoplasmic receptors such as steroid hormone that can be translocated into the nucleus to regulate expression of numerous genes. Membrane receptors possess (i) an extracellular hydrophilic domain, often glycosylated, which recognises the ligand; (ii) a hydrophobic trans-membrane domain that makes embedding possible within the lipid bilayer of the plasma membrane; and (iii) an intra-cytoplasmic domain dedicated to signal transduction within the cell. The binding of a ligand to its receptor is specific, reversible and involves a large number of low-energy bonds (hydrogen, ionic, hydrophobic, and Van der Waals). Thus, at equilibrium, the dissociation rate is equal to the rate of association. Among the receptors of cytokine/growth factors, six types of receptor have intrinsic enzymatic activity (kinase or phosphatase receptors, and guanylyl cyclase-coupled receptors) or not (the G protein-coupled receptors, the receptor-type “channel”, and cytokine receptors).

The ***guanylyl cyclase-coupled receptors*** include natriuretic peptide, nitric oxide, carbon monoxide and enterotoxin receptors. The binding of the ligand to the extracellular domain of its receptor leads to intracellular activation of the guanylate cyclase domain of the receptor chain, and synthesis of a cyclic GMP for activating the cAMP-dependent protein kinase environment [1]. The ***G protein-coupled receptors*** are characterised by seven transmembrane domains. The trimeric G proteins located on the cytoplasmic side of the cell membrane transduce and amplify cell signalling through the production of cyclic AMP. The chemokine receptors are included in this family environment [2]. The ***ion channel linked receptors*** are ligand-dependent ion channels and their opening or closing activities are associated with the nature of the ligand. These receptors can be ionotropic or metabotropic. In the first case, the receptor is actually the pore, and opens following a conformational change made possible by the ligand binding. On the contrary, in the case of metabotropic receptors, ligand-stimulated receptors activate a ligand-independent channel through the intracellular effector environment [3]. ***Cytokine receptors*** can be divided into four groups: (i) receptors with an immunoglobulin-like ectodomain (IL-1α/β, IL-18); (ii) the trimeric members of the TNF receptor superfamily (which include, for instance, RANK, TRAIL receptors and TNF receptors-α/β); (iii), class

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I-cytokine receptors (or haematopoietin receptors) environment [4]; and (iv) class II-cytokine receptors (or interferon and IL-10 receptors) [5]. Class I/II- cytokine receptors have oligomeric structures, where a specific α-chain warrants specific ligand recognition, while one or two channels (β/γ) are used for signal transduction. For instance, the receptors of interleukins (IL) 2, 4, 7, 9 and 15 consist in a specific chain to the cytokine, and the shared IL-2 γ-receptor chain, IL-2 and IL-34 also share a β-receptor chain environment [6]. Similarly, the IL-6 cytokine family (IL-6, IL-11, CNTF, OSM and LIF) shares the gp130 receptor chain environment [7]. Among the cytokine receptor families, some are characterised by intrinsic kinase activity and consequently by their ability for autophosphorylation. They form the receptor tyrosine kinase (RTK) family.

All these receptors tightly control tissue homeostasis, and any dysregulation of these ligand–receptor systems (mutations, overexpression, etc.) disturbs cell communication and leads to pathological situations. Bone formation and bone remodelling are then controlled by a large panel of cytokines and growth factors regulating the dialogue between osteoblasts, osteoclasts and their environment [8]. It has been recognised that cancer cells (bone sarcomas and metastatic cells originating from carcinomas) dysregulate the balance between osteoblasts and osteoclasts, activate osteoclastogenesis and then stimulate bone resorption. Consequently, activated osteoclasts resorb the extracellular bone matrix and release numerous growth factors entrapped in the organic matrix, which stimulate in turn the proliferation of cancer cells. Based on these observations, numerous chemical drugs have been developed to specifically target the various receptor tyrosine kinases activated by mutations, or by the ligands present in the tumour microenvironment. The present review summarises the classification, structure and mechanism, and focuses on the targeting of action of the receptor tyrosine kinases. Their use in the treatment of bone cancers (bone sarcomas and bone metastases) is described and discussed.

2. The receptor tyrosine kinase (RTK) family

2.1. Classification and structure of RTKs

Protein kinases are key enzymes in the regulation of various cellular processes that catalyse the transfer of a phosphate group from ATP to a hydroxyl group of a serine or a threonine. Among the 90 identified genes encoding proteins with tyrosine kinase activity, 58 encode receptors divided into 20 subfamilies [9,10] (Table 1). Of these subfamilies, EGFR/ErbB (class I), the receptor for insulin (class II), for PDGF (Class III), for FGF (class IV), for VEGF (class V) and HGF (MET, Class VI) are strongly associated with oncological diseases. These RTKs are characterised by a single trans-membrane domain and a glycosylated N-terminal extracellular domain with a high number of disulfide bonds. This extracellular domain is involved in the dimerisation process of the receptors, and consequently in ligand recognition (Fig. 1). The composition of these domains (immunoglobulin domains, rich in leucine, lysine and cystein, fibronectin type III domain, etc.) depends on the classes of RTKs and then defines the specificity of the ligands. The RTKs are inserted into the cell membrane thanks to an α-helix trans-membrane domain composed of 20 amino acids. The trans-membrane domain plays a key role in the formation and stabilisation of the dimer of the receptor chains. In the lipid environment of the cell membrane, the α-helices are non-covalently oligomerised [11] (Fig. 1). This type of process makes it possible to pre-dimerise the RTKs in the cell membrane capable of interacting with the corresponding ligand [12].

The cytoplasmic domain harbours a specific domain with tyrosine kinase activity that is involved in the catalysis of the ATP-dependent phosphorylation of receptor chains. It includes two domains: a juxtamembrane region composed of 40–80 amino acids corresponding to the tyrosine kinase domain and a carboxy-terminal region. The tyrosine kinase domain is composed of 12 subdomains organised into two lobes, connected by the kinase insert domain (subdomain V) (Fig. 1). The tyrosine kinase domain includes an activation loop, whose orientation (and phosphorylation) determines the active or inactive state of the kinase domain. The ATP required for kinase activity is housed between the two lobes. The small lobe (named lobe N, for N-terminal, subdomains I–IV), composed of β-sheets and one α helix, binds, stabilises and orients the ATP previously complexed with Mg^{2+} ions. The large lobe (named C, for C-terminal, subdomains VI–IX) is mainly composed of α helices, and plays a part in the chelation of ATP by Mg^{2+} ATP. It then binds the protein substrate containing the tyrosine target and catalyses the transfer of the phosphate group from the ATP to the receptor chains [13]. The size of the tyrosine kinase domain is relatively constant between the different RTKs. On the contrary, the size and content of the juxta- and C-terminal domains vary considerably between the RTK families, conferring the specificity of intracellular signals. For instance, the intracellular domain of PDGFRβ has 552 amino acids and the intracellular domain of EGFR has 542 amino acids, while the FGFR1 shows 425 and TrkA only 356 amino acid residues. The number of tyrosine residues (phosphorylable or not) and their distribution vary significantly between the RTKs. Thus, 27 tyrosine residues are detected for the PDGFRβ (of which 19 can be phosphorylated) and only 11 tyrosines can be detected in TrkA (with 6 phoshorylable tyrosines) [16] . However, a pair of tyrosine residues phosphorylated after RTK activation is found in the activation loop and is required for the functionality of the receptor. The activation of these tyrosine residues stabilises the "open" conformation of the activation loop and both lobes, and also allows the ATP and peptidic substrate environment to bind [13]. An additional, third tyrosine amino acid (located in a close upstream domain) participates in the conformational change of the activation loop. All the mutations on these tyrosine residues result in inactivation of the receptor chains. EGFR is an exception in the RTK families and it has only one tyrosine residue at this position, which is not essential for receptor chain activation and function.

2.2. General mechanism of action

It is admitted that the binding of a dimeric ligand to its receptor chains increases the proximity or/and stabilises the receptor chains that will be then auto-phosphorylated through their kinase domains (a process called trans-phosphorylation). This non-covalent dimerisation is associated with conformational changes that lead to the activation of the cytoplasmic kinase domains of the receptors. In most cases, one of the two receptor chains will trans-phosphorylate specific cytoplasmic tyrosines from other monomeric chain environment [14]. In some cases, the constitutive form of the RTKs is a dimer such as insulin receptors. In addition, some ligands such as EGF are monomeric, and their binding to their receptor induces a conformational change that shifts the intra-molecular loop and exposes a binding domain in the receptor that results in its dimerisation environment [15]. In others, the dimerisation of the ligand is required to activate the receptor chain (i.e., the NGF–TrkA system environment [16]).

In the absence of the ligand, the activation loop self-regulates activation of the receptor because its "closed" conformation inhibits catalytic activity (*cis*-inhibition). Dimerisation of the RTK chains following ligand binding induces the rotation of the N- and