Signalling in the genomic era

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Abstract For a complex organism, short range signalling is not sufficient to coordinate the behaviour of all cells composing itself. The response to stimuli is the reprogramming of cell activity (resulting in differentiation, proliferation, stand by or apoptosis depending on the set of signals). Cells own elaborate and complex systems of proteins that enable them to communicate, including both secreted signalling molecules and related factors, deriving from relic mechanisms. The intra and intercellular signalling are actively studied not only to comprehend the basic mechanisms that allowed the evolution of mammals species on earth, but also because the alteration of one or more of these pathways is recognized to be involved in a crescent number of human diseases, both degenerative and tumoural. That is, a growing body of evidences suggest that every human disease may be analyzed and classified by a “signalling disease” point of view. This approach opens new therapeutic perspectives, virtually amplifying for every single disease the number of therapeutic targets (in terms of both genes and proteins) to upstream and/or downstream, short and/or long distance proteins interacting with the altered molecule, thus individuating many other targets to which act upon.

Keywords Signalling · Etiopathogenesis · Degeneration · Neoplasm

Cells in multicellular organisms undergo to a “social control”, being exposed to a wide number of different signals

coming from the external and/or internal environment. Intercellular signalling is a complex, intricate machinery that allows cells to determine their reciprocal position, specialised role and cycle progression. Cells have developed elaborate and complex systems enabling them to communicate, including both secreted signalling molecules and related factors, such as receptors, enzymes, GTP-binding proteins and other interacting proteins. Higher animal cells communicate by means of hundreds signalling molecules such as small peptides, proteins, amino acids, nucleotides, steroids, retinoids, fatty acids and derivatives, gases such as nitric oxide (NO) and carbon monoxide (CO). In response to specific stimuli, every cell modulates its activity, resulting in differentiation, proliferation, stand by or apoptosis depending on the set of signals (Morgan 1989). In the presence of specific receptors, the cell is tuned to catch signals. Accordingly to the intracellular machinery it possesses, the cell will be able to interpret and integrate the received information. The same signalling molecule may bind slightly different type of receptors and induce different effects on different target cells. Also binding the same receptor, a signalling molecule may induce different effects, because receptors maybe coupled to different responsive intra cellular machinery. Thanks to this complex organization, cells continuously adapt to environmental changes. These systems probably derive from relic mechanisms enabling unicellular organisms to influence the behaviour of neighbouring cells, as observed in *Saccharomyces cerevisiae* in preparation for sexual mating. Signal transduction pathways had been actively studied to understand the basic mechanisms that allowed the evolution of species on earth. As an example, studies of oncogenes and proto-oncogenes have contributed to introduce us to the molecular mechanisms underlying cancer, and also have uncovered many previously unknown proteins involved in signalling pathways (Alberts et al. 2007). Moreover, evidences have highlighted that the alteration of

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one or more of these pathways is involved in a growing number of human diseases, both degenerative and tumoural (Tan et al. 2009; Duyckaerts et al. 2009; de Jager et al. 2009). That is, every human disease might be seen as a “signalling disease”. Intra and inter cellular signalling is strictly regulated (Morgan 1989). Molecular biology methodologies have revolutionized the study of gene expression and the ability to characterize protein structure and interactions. Special improvement was observed in analyses of low expressed intra/extracellular or membrane exposed proteins, whose quantity may be amplified *in vitro*. Gene mutation, mRNA expression and proteomic studies look inside the basic mechanisms of regulation along the cell cycle phases. Signals among cells comprise a number of molecules undergoing continual turnover. Their production, catabolism, renewal, turnover rate and promptness in the response are strictly controlled by a complex mechanism of gene expression regulation. In fact, the “social control” which cells undergo to becomes evident when it fails, as in degeneration or in tumours. As an example, phosphatidylinositol lipids (PI) signalling stimulated the fervour of researchers inasmuch potentially involved in a growing number of diseases. The first observation suggesting a role for PI in signal transduction was done in 1953, describing that extracellular signals stimulated the incorporation of radioactive phosphate into cell membrane phosphatidylinositol (Sekar and Hokin 1986; Berridge 1993; Ferris and Snyder 1992; Meldolesi 1992). Later it was described a phosphatidylinositol cycle in the nucleus, distinguished from the cell membrane inositol cycle (Irvine 2002). Among the molecules involved in PI signalling, of interest are phosphoinositide-specific phospholipase C (PI-PLC) enzymes. In less than a second, PI-PLC cleaves PIP₂ into inositol trisphosphate (IP₃) and diacylglycerol (DAG). IP₃, a small water-soluble molecule, diffuses rapidly to the cytoplasm. IP₃ induces calcium release from the endoplasmic reticulum (ER) by binding to IP₃-gated calcium-release channels located in the ER membrane. It is well known that calcium is a crucial mediator in many activities, both in cells and tissues. The initial calcium increase induced by IP₃ propagates as a wave through the cytoplasm (Hisatsune et al. 2005). DAG exerts two potential signalling roles. First, it can be further cleaved to release arachidonic acid, which either acts as a messenger or is used in the synthesis of eicosanoids. That is, this signalling pathway is involved in inflammation, as recently suggested by *in vitro* studies on astrocytes activation (Lo Vasco et al. 2010). Second, DAG activates a serine/threonine calcium dependent protein kinase (PKC) (Noh et al. 1995). The increase in calcium levels induced by IP₃ moves PKC to translocate from the cytoplasm to the plasma membrane cytoplasmic face, where it is activated. PKC phosphorylates specific serine or threonine residues on target proteins. Remarkably, cAMP and calcium intra cellular signalling pathways interact at several

levels in the hierarchy of control, because calcium and cAMP levels can influence each other. Some of the enzymes acting upon cAMP, such as phosphodiesterases and adenylyl cyclases, are regulated by complexes including calcium. Conversely, A-kinase phosphorylates calcium channels and pumps altering their activity. Phosphorylation of the ER receptor of IP₃ either inhibits or promotes IP₃-induced calcium release, depending on the cell type. Moreover, enzymes regulated by calcium and cAMP influence each other and also some kinases are phosphorylated by A-kinase. Finally, these enzymes have interacting effects on shared downstream target molecules, such the cAMP response element-binding (CREB) gene (Suh et al. 2008). Accordingly to what is known for hormones, maybe also for PI-PLCs a double response induction exists after a specific activation stimulus. The direct induction of the transcription of a small number of specific genes (within maximum 1 h) (primary response) produces proteins which in turn activate other genes and produce a delayed (secondary) response. Being related to the pathways of PKCs, of Mitogen Activated Protein Kinase (MAPK/ERK) and CREB, PI-PLCs seem involved in the progression of various tumours (Cocco et al. 2009; Lo Vasco et al. 2004). The suggested involvement of PI-PLCs in inflammatory activation of glia (Lo Vasco et al. 2010) seems also related with tumour progression (Ikuta et al. 2008). The nature and the meaning of this involvement are still unclear. Moreover, PI-PLCs have been analyzed for their possible role in the etiopathogenesis of mental illnesses, such as major depression (Pandey et al. 1999) and schizophrenia (Lin et al. 1999; Ríková et al. 1997), indicating a possible role in neurotransmission alteration. Further interesting data arise from the observation that calcium increase, in which PI-PLCs are involved, is crucial in the retinal degeneration observed in many diseases, such as retinitis pigmentosa, macular degeneration and diabetic retinopathy (Lee et al. 2003). In visual transduction, receptors are activated by light, which leads to cGMP decrease. Light stimulation causes hyper polarization of the plasma membrane leading to the closure of the sodium channels (Liu et al. 2009). The closure inhibits the influx of calcium, causing its concentration to fall. This mechanism allows the adaptation, that is the photoreceptor to revert quickly to its resting state (Liu et al. 2009). Another active research field had been represented by the heart. Studies highlighted that some PI-PLC isoforms influence cardiac function. The total sarcolemmal PI-PLC activity was decreased in ischemia and increased upon reperfusion, with differential changes in some isoforms being detected (Asemu et al. 2003). For this reason, PI-PLC is considered a potential target for cardioprotection during oxidative stress (Tappia et al. 2010). Resuming, as other signalling pathways, inositol phospholipids' is crucial for and overlaps in many functions, so that its alteration might be involved in diseases which appear very different for etiopathogenesis and/or clinical

epiphenomena. As far as one can see, the lesson of the basics of signalling is that the same pathway alteration might underlie to different diseases. The most important consequence is to consider not the single molecule, but all the related components of the pathway the molecule belongs to. This might allow the identification of many other targets for diagnosis, prognosis and therapy. That is, for every disease the disrupted pathway as a whole might virtually amplify the number of target molecules (genes, mRNAs and proteins) to upstream and/or downstream, short and/or long distance interacting proteins. This approach looks at diseases as signalling pathologies depending on the involved pathways. On the other hand, apparently different diseases share common signalling aspects. As an example, the analysis of PI's signalling related molecules would be useful both in degenerative and tumoural diseases. Deletions of PI-PLC genes are active research targets both in schizophrenia and in various tumours. Moreover, abnormal expression of PI-PLC proteins stimulates the interest both in retinal degeneration and in myocardial ischemia. At now these studies are directed to identify the potentiality of such molecules for diagnosis and/or prognosis. Maybe future perspectives will be directed to use them for therapeutical approach.

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