The usefulness of post-genomics tools for characterization of the amine cross-talk in mammalian cells

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Abstract
Evidence is growing in favour of a relationship between cancer and chronic inflammation, and particularly of the role of a polyamine and histamine metabolic interplay involved in these physiopathological problems, which are indeed highly complex biological systems. Decodification of the complex inter- and intra-cellular signalling mechanisms that control these effects is not an easy task, which must be helped by systems biology technologies, including new tools for location and integration of database-stored information and predictive mathematical models, as well as functional genomics and other experimental molecular approaches necessary for hypothesis validation. We review the state of the art and present our latest efforts in this area, focused on the amine metabolism field.

A brief overview of amine metabolism in immune system and cancer cells
Decarboxylation of amino acids produces biogenic amines that play important biosignalling roles in mammalian cells. Those derived from arginine/ornithine (polyamines) are essential for proliferation in every living cell (especially in cancer and other highly proliferative cells and tissues), as explained elsewhere in this issue of Biochemical Society Transactions. Histamine (the product of the histidine decarboxylase reaction) is mainly synthesized in immune cells (mast cells, basophilic, macrophages, platelets, T-cells and dendritic cells), where it is considered as a proinflammatory mediator [1]. Mast cells, the major producer of histamine in the human body, are bone-marrow-derived cells expressing a variety of phenotypic features as determined by the local environment. They are located in connective tissue (skin and peritoneal cavity) and mucosa, and synthesize inflammatory mediators released to the environment in response to different stimuli [2]. Some of these mast cell mediators are accumulated into granules and released by exocytosis, as is the case with specific proteinases (tryptase) and histamine. Histamine is synthesized by HDC (histidine decarboxylase) [3]. Other immune cells (for instance, macrophages) produce but do not store histamine into granules. Of course, these immune cells also produce polyamines, as essential compounds for macromolecular synthesis and cell survival. Thus they can be considered as complex amine-producing and amine-handling cell models.

Nowadays, clear relationships are being established between chronic inflammation and several types of tumours [4]. Histamine- and polyamine-producing cells are often close in vivo (for instance, during carcinoma and/or leukaemia growth), and malignant forms of histamine-producing cells have been reported (mastocytosis, basophilic leukaemias and some types of gastric cancer, among others) [5–8]. Specific roles of histamine and polyamines in the cross-talk between mast cells and tumour cells have been reported [9]: histamine can promote or inhibit tumour growth depending on the receptor expressed by the target tumour cell type. In turn, polyamines and their oxidized products are related to the mast cell proliferation/death equilibrium and the liberation dynamics of mast cell granule mediators to the medium [9,10].

Working on murine mast cell models, we have previously observed antagonistic relationships between histamine and polyamine metabolisms [11,12]. We also observed that mast cells are particularly sensitive to the apoptosis inducers produced from polyamines by serum amine oxidases [10,13]. All of these facts are particularly interesting for gut physiology, since diet can provide compounds (i.e. drugs, biogenic amines, enterobacterial products, etc.) affecting mast cell proliferation/death and, consequently, mast cell roles in gut inflammatory and neoplastic diseases.

From a biochemical point of view, interferences between histamine and polyamine metabolic pathways are not surprising, since both types of biogenic amines share structural properties and common metabolic steps, as observed with purified enzymes or animal cultured cell models (Figure 1) [14]: (i) histamine interferes with spermine transport systems and has a co-operative effect with inducers of the key enzyme...
of polyamine degradation, SSAT (spermine spermidine N\(^1\)-acetyltransferase), so reducing the intracellular polyamine content in mouse C57 mast cells [12]; (ii) some diamines inhibit the uptake of cationic amino acids by the y\(^+\) system, as observed in Ehrlich ascitic tumour cells [15]; (iii) polyamines and histamine can be covalently cross-linked to proteins by the action of transglutaminases [16]; (iv) some amino oxidases degrade both polyamine- and histamine-producing toxic products (aldehydes and oxygen free radicals) [17]. In addition, SAM (S-adenosylmethionine), the aminopropyl donor for both spermidine and spermine synthesis, is also required for methylation of histamine, as a previous step for degradation in many cell types [1]. Since SAM is also a methyl donor for DNA methylases, it could constitute a metabolic node connecting epigenetic response and amine metabolism (among other pathways). Experimental evidence for a relationship between DNA methylation and both polyamine and histamine metabolisms, has also been reported [18,19].

**The physiopathological problem: a highly complex biological system**

At the molecular level, the picture of the problem in vivo is extremely complex [20]. As stated in the first updated report of COST Action 922, many proteins (enzymes, receptors and transporters) are involved in the amine cross-talk between inflammatory and cancer cells [21]. In addition, many other elements involved in intercellular communication and transduction signal elements, having a high degree of tissue specificity, should be characterized and taken into account.
Figure 2 | Intercellular communication among mast cells, cancer cells and endothelial cells

On the left, paracrine signal mediators positive for tumour progression; on the right, paracrine signal mediators negative for tumour progression. The processes affected by the mediators are specified within parentheses. Arrows go from the mediator-synthesizing cell to the target cell. Continuous lines, effects on tumour cells; dotted and dashed lines, effects on mast cells; dotted lines, effects on endothelial cells.

Positive effects on tumour progression

Negative effects on tumour progression

Histamine, tryptase and proteoglycans have been described as essential for mast cell differentiation [22,23], and as both angiogenesis and tumour progression modulators [24,25]. Mast cell degranulation is followed by secretion of different interleukins and growth factors that also can affect cancer cell growth (and polyamine metabolism) even with opposite roles: mast cells have therefore been described as ‘Dr Jekyll and Mr Hyde’ for tumour growth [9]. Of course, the effects elicited by these factors are dependent on the signal transduction pathways operative in the target cells. Mast cells also produce invasiveness factors (for instance, matrix metalloproteinases and other proteases), since they need these activities to infiltrate into tissues during the final stages of their maturation process. Nevertheless, these proteins could also help in tumour invasion. From these findings, it is deduced that another cell type must be added to this picture: the endothelial cell, responsible for new vessel formation (angiogenesis), an essential process for tumour survival, invasion and metastasis [26]. From the same proteinogenic precursor as polyamines, arginine, endothelial cells and some tumours can produce nitric oxide, described as a regulator of amine metabolism, inflammation and cell proliferation [27].

The present degree of knowledge was reached with an enormous quantity of work necessary for molecular characterization of individual elements and metabolic pathways, as well as for observations of biological effect. However, all the useful information that could contribute to complete the picture has been distributed between different research areas (for instance, immunology, oncology, cardiovascular and basic molecular biology and biochemical research), which makes it more difficult to find its location, and consequently restricts the perspective for formulation of further experimental hypotheses.

The need for post-genomics tool development

During the few last years, we have been asking for more holistic approaches to characterize this highly complex biological system [21,28]. The information generated by Genome Projects, as well as the results of many molecular and functional genomics projects, is being accumulated in different public databanks and bibliographies. It provides an important source of knowledge which needs the development of more efficient tools to be located and analysed properly. It is clear that biomathematics and bioinformatics (that is, systems biology technologies) could help to reach this goal. The most recent
bibliography shares this analysis of the situation and supports this assessment [20,29–32].

Figure 3 shows a scheme of our proposal for combined biocomputational and experimental approaches in order to gain efficiency in the advance of a more systemic knowledge of amine metabolism. More details on this proposal can be obtained from the website http://www.asp.uma.es. In this sense, we have worked on different action lines. Metabolic modelling allows us to predict the limiting step(s) and the more efficient way(s) for intervention under a given metabolic state. Using this hypothesis, the first mathematical predictive model for polyamine metabolism in mammals has been developed and validated [33]. New biomodules, for instance, arginine [34], histidine and methionine pathways, as well as oxidative metabolism (as the source of acetyl-CoA) (Figure 2) could be added to this first one, in order to get a more integrated metabolic view of the amine metabolism. This approach would indeed be helped by development of improved methods for text mining, since most of the kinetic constants are reported in the literature.

With respect to both intercellular communication and intracellular signalling mechanisms that control gene expression, they could be decoded using a combination of graph-theory-based interactomics studies and functional genomics approaches. This approach needs to be assisted by automated tools for integration of the information stored in data banks (for instance, promoter cis elements, experimentally assessed macromolecular interactions, and experimental functional genomics results on specific species, tissues and physiopathological circumstances). At present, we are also working on different projects on the development of new biocomputational tools for these purposes [35].

Nevertheless, some specific hypotheses derived from in silico predictions on amine-related genes could need further experimental validation. We advanced the generation of a cDNA macroarray of human amine-related probes for simultaneous detection of gene expression alterations caused by a given stimulus. At present, the array contains cDNA probes for the most important proteins of the following metabolic pathways: polyamines, histamine metabolism (including receptors), arginine (including urea cycle enzymes and inducible nitric oxide synthase), methionine cycle and other inflammation, cell migration and proliferation-related probes, many of them mentioned in Figures 1 and 2. This first array is being validated on human mast cells, a cell type with an important lack of information concerning its intracellular signalling network, and for which functional genomics studies have not been frequent so far [36–38]. Nevertheless, the array is continuously growing and could be applied to many other human cell types and physiopathological conditions.

In any case, once a specific macromolecular element (protein or nucleic acid) is located as the best target for intervention in a given pathway, a deeper characterization of structure–function relationships of this macromolecule can also be assisted in silico by macromolecular modelling and molecular dynamics calculations that could provide valuable insight for design of new and more specific structural and functional modulators (Figure 3) [39].

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