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Development of an expression macroarray for amine metabolism-related genes

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Summary. Cationic amino acids are the precursors of biogenic amines, histamine from histidine, and putrescine, spermidine and spermine from arginine/ornithine (and methionine), as well as nitric oxide. These amines play important biological roles in inter- and intracellular signaling mechanisms related to inflammation, cell proliferation and neurotransmission. Biochemical and epidemiological relationships between arginine-derived products and histamine have been reported to play important roles in physiopathological problems. In this communication, we describe the construction of an expression macroarray containing more than 30 human probes for most of the key proteins involved in biogenic amines metabolisms, as well as other inflammation- and proliferation-related probes. The array has been validated on human mast HMC-1 cells. On this model, we have got further support for an inverse correlation between polyamine and histamine synthesis previously observed on murine basophilic models. These tools should also be helpful to understand the amine roles in many other inflammatory and neoplastic pathologies.

Keywords: Expression macroarray – Polyamines – Histamine – Mast cells – Inflammation

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDC, Histidine decarboxylase; HMT, histamine N-methyl transferase; iNOS, inducible nitric oxide synthase; SAM, S-adenosyl-methionine; SMS, spermine synthase; SRM, spermidine synthase; TPS, tryptase

Introduction

Cationic proteinogenic amino acids are also the precursors of different compounds that play important biosignaling roles in mammalian cells. Nitric oxide synthases produce nitric oxide from arginine, a gas that plays pleiotropic roles in inflammation, angiogenesis and cell proliferation (Lechner et al., 2005; Montañez et al., 2007). Arginase activities hydrolyse the guanidinium group of arginine to produce urea and ornithine. Putrescine is the ornithine alpha-decarboxylation product. In plants and bacteria, agmatine is obtained by the arginine decarboxylase activity, but this possibility in mammals is still controversial. In any case, diet and intestinal flora can act as agmatine sources for animals. The removal of the guanidinium group from agmatine (by the agmatinase activity) can also produce putrescine. The addition of one or two terminal aminopropyl groups from decarboxylated S-adenosyl-methionine to putrescine produces higher polyamines, named spermidine and spermine, respectively. Characteristics of these metabolic pathways and the physiopathological roles of polyamines have been recently reviewed in this (see this issue, for instance) and other journals (Agostinelli et al., 2004; Gerner and Meyskens, 2004; Janne et al., 2005; Seiler and Raul, 2005; Pegg, 2006).

Histamine is the product of the histidine decarboxylase activity (HDC) (Moya-García et al., 2005). This enzyme is expressed in a short set of mammalian cell types, including immune cells (mainly in mast cells), gastric enterochromaffin-like cells and histaminergic neurons. The degradation of these amines can include the action of different transfererases (as it is the case of spermine/spermidine acetyltransferase and histidine methyl-transferase) and different types of amine oxidases (Winterkamp et al., 2002; Seiler, 2004). Thus, amine metabolic pathways are convergent in some activities able to reduce the levels of the intracellular free-amine pool, namely transglutaminase and diamino oxidase activities (Ballas et al., 1985; Seiler, 2004).

Amines derived from arginine/ornithine (and methionine), are essential for proliferation in every living cell,

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especially in cancer and other highly proliferative cells and tissues (Gerner and Meyskens, 2004). The roles of polyamines in other physiopathological processes, including inflammation-related diseases and neurological functions, have also been proposed (Furumitsu et al., 2000). It seems that polyamines mainly play their biological roles by direct binding to nucleic acids and intracellular proteins (Igarashi and Kashiwagi, 2000); however, specific binding to membrane receptors and/or transport systems have also been reported (Masuko et al., 2003; Turecek et al., 2004; Hoshino et al., 2005).

Histamine is mainly considered as a proinflammatory mediator, but also plays important roles in neurotransmission and neuroendocrine behaviour. It binds to different tissue-specific proteins, for instance, the different histamine receptor subtypes, the organic cation transport systems Oct2/3, and the vesicular amine transporters (Weihe and Eiden, 2000; Dy and Schneider, 2004; Akdis and Simons, 2006; Ogasawara et al., 2006) to send intracellular signals or to move among the different intracellular compartments. In addition, due to their cationic nature and structure, both kinds of biogenic amines are able to bind DNA (Ruiz-Chica et al., 2001, 2006), so they could be direct modulators of nucleic acid synthesis and gene expression.

This intertwound metabolic map of biogenic amines is controlled by signaling networks, having many cell-type specificities that are responsible for the biological roles of these compounds in different tissues and for the pathological consequences of their alterations. In fact, NO-, histamine- and polyamine producing cells are often close *in vivo* (for instance, endothelial, immune and cancer cells during carcinoma and/or leukemia growth). Clear relationships have been established between chronic inflammation and several types of tumors (Coussens and Werb, 2002) with proposed roles for histamine and polyamines (Medina et al., 2003; Theoharides and Conti, 2004). There also are neoplastic models that synthetize both histamine and polyamines (for instance basophilic leukemias and malignant mastocytosis) (García-Montero et al., 2006; Krauth et al., 2006). All these pieces of information together suggest that the cationic amino acid product interplay must be relevant not only for cancer progression and immune response, but also for other physiopathological processes, as hepatic regeneration, cardiovascular and male-fertility problems and neurological and neuroendocrine-related ones (Medina et al., 2003).

It is evident that the picture at molecular level is extremely complex *in vivo* for any of the physiological problems mentioned above. Post-genomics tools and approaches can help to obtain, organize and discuss easier the related information, and thus improve the effectiveness in the advance of knowledge, and consequently the yield of research investments. We have been claiming for more holistic approaches to characterize amine metabolism and its physiological roles at molecular level (Medina et al., 2005). They should take advantage of the Bioinformatics (Medina et al., 2005; Rodríguez-Caso et al., 2005, 2006) and functional genomics advances.

In this communication, we describe the generation of a cDNA macroarray of human amine-related probes for simultaneous detection of gene expression alterations caused by a given stimulus on different human cell types. At present, the array contains cDNA probes for the most important proteins of polyamine metabolism, histamine metabolism (including histamine receptors), arginine metabolism (including urea cycle enzymes and inducible nitric oxide synthase, iNOS), methionine cycle and other inflammations, cell migration and proliferation-related probes. In the present work, we describe the strategy for the array construction and its validation with human mast HMC-1 cells at different proliferation stages. The expression array can be useful for characterization of many other human amine-producing and amine-handling cells and tissues under many other physiopathological conditions. The array is continuously growing and open for collaboration with other research groups.

Materials and methods

Cell cultures and treatments

The human mast cell line HMC-1 was kindly supplied by Dr. J. Butterfield (Mayo Clinic, Rochester). As the routine procedure, they were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 at a starting density of 1×10^5 cells/ml in Iscove's medium (Biowhitaker) supplemented with 10% calf serum (PAA, Austria), 2 mM L-glutamine, 50 IU/ml penicillin + 50 IU/ml streptomycin (Biowhitaker), 1.25 µl/ml Fungizone (Gibco), iron supplement (Sigma) and 1.2 mM α-thioglycerol (Sigma). For the present experiments, at day 5, they were pelleted and resuspended in fresh medium with 2.5% calf serum. At different stages of the culture process, cells were harvested and counted in a Neubauer counting-chamber after staining with Trypan blue. Only unstained cells were considered. Each cell number value is the mean of 4 different determinations.

Array preparation

A complete list of cDNA fragments, including two internal controls, beta-actin and glyceraldehyde-3-phosphate dehydrogenase cDNAs is shown in Table 1. Most of them have an average size ca. 450 bp and were obtained by RT/PCR or PCR (depending on the template origin), and then cloned into a pGEM-T easy vector (Promega). To be used as probes, the fragments were amplified by PCR with specific primers (sense and antisense, respectively, to the terminal 17–24 bases of the sequences specified in Table 1). PCR probe amplification was carried out using the following program: 95 °C for 5 min, 35 cycles of 95 °C for 30 s, annealing temperature for 30 s

Table 1. Probes used for amine-related macroarray

Probe	Abbr.	Gen ID	Sequence range (bp)	Origin
Arginine metabolism				
Arginase 1[c]	ARG1	NM_000045	564–1018	RT/PCR from human liver
Carbamoyl-phosphate synthase[c]	CPSI	NM_001875	1579–1821	RT/PCR from human liver
Arginase 2[c]	ARG2	NM_001172	155–588	RT/PCR from human kidney
Ornithine carbamylase[c]	OTC	NM_000531	115–630	RT/PCR from HMC-I
Methionine metabolism				
Cystathionine beta-synthase[c]	CBS	NM_000071	94–579	RT/PCR from human liver
Methylenetetrahydrofolate reductase[c]	MTHFR	NM_005957	596–1148	RT/PCR from human liver
Cystathionine gamma-lyase[c]	CTHI	NM_001902	495–1018	RT/PCR from human liver
Polyamines metabolism				
Ornithine decarboxylase[c]	ODC	NM_002539	413–1008	Dr. Janne (Helsinki, Finland)
Agmatinase[a]	AGM	NM_024758	856–1395	Dr. Morris (Pittsburgh, USA)
S-Adenosyl methionine decarboxylase[d]	SAMDC	NM_001634	197–737	RT/PCR from HMC-I
Spermidine synthase[c]	SRM	NM_003132	415–931	RT/PCR from HMC-I
Spermine synthase[c]	SMS	NM_004595	259–799	RT/PCR from HMC-I
Spermidine/spermine acetyltransferase[c]	SAT	NM_002970	181–673	Dr. Casero (Baltimore, USA)
Polyamine oxidase 1[a]	PAOh1	NM_152911	641–1311	RT/PCR from HMC-I
5′-Methylthioadenosine phosphorylase[c]	MTAP	NM_002451	145–672	RT/PCR from HMC-I
Histamine metabolism				
Histidine decarboxylase[a]	HDC	NM_002112	103–664	RT/PCR from HMC-I
Histamine N-methyltransferase[c]	HMT	NM_001024074	68–646	Dr. Weinshilboum (Minnesota, USA)
Histamine H1 receptor[c]	H1R	NM_000861	790–1297	Dr. De Backer (Beerse, Belgium)
Histamine H2 receptor[c]	H2R	NM_022304	633–1128	RT/PCR from HMC-I
Histamine H4 receptor[c]	H4R	NM_021624	481–986	RT/PCR from HMC-I
Other genes				
Vascular endothelial growth factor[b]	VEGF	NM_001025366	1701–1896	RT/PCR from HMC-I
Vascular endothelial growth factor C[d]	VEGFc	NM_005429	605–904	RT/PCR from HMC-I
Myc proto-oncogene	cMYC	NM_002467	938–1471	Dr. Lozano (Malaga, Spain)
Cell division control protein 42 homolog[d]	CDC42	NM_001039802	45–653	RT/PCR from HMC-I
Integrin alpha-5[d]	INTα5	NM_002205	3045–3215	RT/PCR from HMC-I
Tumor necrosis factor alpha[d]	TNFα	NM_000594	890–1332	RT/PCR from HMC-I
Nitric oxide synthase, inducible[d]	iNOS	NM_000625	390–943	Dr. Shapiro (Pittsburgh, USA)
Hypoxia-inducible factor 1 alpha[c]	HIF1α	NM_001530	2284–2727	RT/PCR from hypoxic HUVEC
Cyclooxygenase 2[d]	COX2	NM_000963	611–915	RT/PCR from PMA-treated HUVEC
Tryptase[d]	TPS	NM_003294	325–750	RT/PCR from HMC-I
Peroxisome proliferator-activated receptor gamma[c]	PPARg	NM_015869	1120–1595	RT/PCR from human liver
Controls				
Glyceraldehyde-3-phosphate dehydrogenase[d]	GAPDH	NM_002046	197–801	RT/PCR from human blood
Beta-actin[d]	βACT	NM_001101	635–1157	RT/PCR from HMC-I
pGemT easy	pGTe	–	–	PROMEGA®

Annealing temperature used for PCR amplification of probes: [a] 53 °C; [b] 55 °C; [c] 57 °C; [d] 60 °C

and 72 °C for 50 s, 72 °C for 7 min. Empirically, four different annealing temperatures were determined for simultaneous amplification of the probes (see Table 1). The amplification of a single band with the correct size was confirmed in all the cases, and its sequence was checked by double-strand sequencing (Secugen, Spain). Custom-made arrays were robotically printed on Hybond-N^+ membranes (GE Healthcare, UK) by Newbiotechnics (Spain), by using 20 ng of cDNA for each probe spot by using a Biomek 2000 Workingstation, (Biomek, USA). Three spots for each probe were printed, as well as for two synthetic sequences with no counterpart in the human genome (provided by Newbiotechnics) that were used as internal controls during the reverse transcription and the labelling of the samples. After hybridization, the result for a single messenger is considered as the mean of its 3-spot intensities.

Sample preparation

The RNA samples were isolated with the GenElute Mammalian Total RNA Kit from the cell cultures mentioned above. They were spectrophotometrically quantified. Only samples with absorbance ratios (260 nm/280 nm) around 1.9 were used. For each sample, a ^{32}P-labeled target cDNA mixture was synthetized from 4 µg of total RNA and the reagents provided with the transcriptor/1st Strand cDNA Synthesis Kit for

RT/PCR (AMV) (Roche), in a final reaction volume of 50 µl, containing random primers, a mix of dATP, dTTP and dGTP (0.5 nM, final concentration) and 50 µCi [α-^{32}P]-dCTP (GE Healthcare, UK). Products were purified with Sephadex G50 (SIGMA) columns to remove the unincorporated nucleotides and then denatured at 95 °C before hybridization. After prehybrization for 3 h at 65 °C, nylon membranes were hybridized overnight at 65 °C with the ^{32}P-labeled cDNA in hybridization buffer Church's buffer (0.5 M sodium phosphate buffer, pH 7.2, 1% BSA, 7% SDS, 1 mM EDTA). They were finally washed three times during 15 min at 65 °C in 40 mM sodium phosphate buffer pH 7.0 supplemented with 0.1% SDS. Signals were detected by an Imagining Plate (Fujifilm). Signal intensities from the images were quantified using the software ImageGauge (Fujifilm). Only trends observed as highly repetitive in more than 3 independent experiments are mentioned.

Alternative tests for single messengers

For Northern blots, 20 µg of total RNA was separated on 1% agarose gel containing 1% formaldehyde, and then transferred to a nylon membrane. ^{32}P-labeled probe cDNA was prepared with a HighPrime labeling kit (Roche). The protocol used for hybridization and detection was similar to that used for the macroarray analysis, except for the hybridization buffer composition (formamide 50%, Denhardt's reactive 5×, SSPE 5×, SDS 1%, DNA 20 µg/ml) and incubation temperatures (42 °C). Other details have been described previously (Urdiales et al., 1992; Fajardo et al., 2001). HDC messenger was quantified by the real time RT/PCR method reported previously (Melgarejo et al., 2006). Triptase mRNA was also quantified by real-time RT-PCR using the following primers: forward: 5′-CCCAGTGG GTGCTGACCGC-3′ and reverse: 5′-GCGGTCAGCACCCACTGGG-3′. The program profile used for tryptase amplification was: 95 °C for 5 min and 40 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 55 °C and extension for 1 min at 72 °C. The tryptase signal was normalized to signal obtained from beta-actin amplification in the same sample using the program: 95 °C for 10 min and 28 cycles of denaturation for 30 s at 95 °C, annealing for 30 s at 57 °C and extension for 1 min at 70 °C on a MX-3000 thermal cycler (Stratagene). Primers for beta-actin amplification were those described in Table 1. The ratio of expression was calculated according to the method described by Liu and Saint (2002).

Results and discussion

In this pilot project, we have chosen representative probes for several metabolic modules closely related to cationic amino acid metabolism and their proposed phsyiological roles. Table 1 shows the present state of the array with respect to the name and function of their elements. Arginine is the proteinogenic precursor for polyamine synthesis and one of the major nitrogen vectors among mammalian tissues (Márquez et al., 1989). Urea cycle probes can be specially interesting for studies with arginine-synthetizing human models (for instance, hepatomas or regenerating liver, and kydney pathologies) (Craig et al., 2006). The agmatinase activity and/or its substrate agmatine have been reported as important molecules for gut, hepatic, kidney and brain physiology (Reis and Regunathan, 2000; Morris, 2003, 2004). The array also contains probes for most of the relevant enzymes for regulation of polyamine metabolism (Urdiales et al., 2001).

Among the probes related to histamine metabolism, the presence of those for different histamine receptors could reveal some tissue specificities, since they are differentially expressed by different cell types, so determining the effects elicited by histamine on the target cells, which are sometimes antagonic signals for cell proliferation and differentiation (Hill et al., 1997; Akdis and Simons, 2006; Ogasawara et al., 2006). Methionine cycle enzymes, that is S-adenosyl-methionine (SAM)-related enzymes, are interesting not only for spermidine and spermine synthesis, but also for histamine degradation as substrate of histamine N-methyl transferase (HMT). SAM recycling mingled with folate cycle are the biosources for DNA methylation and, consequently, play essential roles in epigenetic responses. Intracellular polyamine levels have been described as modulators of DNA methylation in mammalian proliferating cells (Heby et al., 1988). Histamine decarboxylase expression is also regulated by methylation/demethylation of its promoter (Kuramasu et al., 1998; Suzuki-Ishigaki et al., 2000). Genes clearly related to cell migration and angiogenesis were also included (i.e.: integrins and vascular endothelial growth factors) (Muñoz-Chápuli et al., 2004); these processes are required for mast cell survival and infiltration within tissues, as well as angiogenesis and tumor invasion and metastasis (Quesada et al., 2006). Other genes could be the indicators of the cell death/survival signaling (i.e.: c-myc and TNF-alpha). C-myc has been proposed as an inducer of leukemia cell apoptosis (Tiberio et al., 2001). TNF-alpha is a proinflammatory factor essential for mast cell development (Wright et al., 2006). Tryptase is a specific mast cell mediator stored in a coordinated manner to proteoglycanes and histamine in mast cell granules (Hallgren and Pejler, 2006). Tryptase stimulates proteinase activated receptors and consequently the inflammatory response (Theoharides and Conti, 2004). In a recent *in silico* study on the human transcription factor network, we detected that the peroxisome proliferation receptor gamma (PPAR-gamma) could be a connector element between inflammation-related and proliferation-related transcription factors (Rodríguez-Caso et al., 2005). More specifically, it has been described as a regulator of SAT expression (Babbar et al., 2003). Nitric oxide synthase is proposed together with oxidized polyamines as a mast cell degranulation inhibitor (Theoharides and Conti, 2004). Hipoxia-inducible factor 1-alpha and cyclooxygenase-2 are two gene products clearly related to stress and inflammatory responses (Fajardo et al., 2004; Meric et al., 2006; Tsatsanis et al., 2006). Summarizing, the array, in its present state, contains key probes for human cell proliferation, inflammation and angiogenesis-

related processes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin probes were considered as housekeeping genes for signal normalization purposes.

The generation of the array involved a complex iterative process of primer design, template (RNA or DNA) collection and amplification reaction improvements to get a single amplification band for every case, as well as further subcloning and sequencing processes. It was our aim to keep probes within a range of melting temperatures ($89 \pm 5\,^{\circ}C$) and probe lengths (around 450 nucleotides for most of them, see Table 1) trying to facilitate the final obtention process and to get more comparative results among the expression levels of simultanously detected messengers from a same sample.

We have chosen human mast cells as a first model for validation of the array, due to the following reasons: i) Mast cells are bone-marrow derived cells expressing a variety of phenotypic features within tissues as determined by the local environment. Mature mast cells are located in connective tissue (skin and peritoneal cavity) and mucosa close to blood vessels (Nathan, 2002). During their migration from bone-marrow to their final location, they must overcome different stress situations that involve migration through blood vessels and tissues, and drastic changes in the environment composition (including oxygen). Thus, important changes in stress-, adhesion- and migration-related genes could be suspected along their life-cycles. ii) Mast cells synthetize inflammatory mediators released to the environment in response to different stimuli (Metcalfe et al., 1997). Some of these mediators are accumulated into granules and released by exocytosis, as it is the case of specific proteinases (tryptase) and histamine. Histamine is synthetized by histidine decarboxylase (Moya-García et al., 2005). Both enzymes, tryptase and histidine decarboxylase (HDC) have been described as essential for mast cell differentiation (Ohtsu et al., 2001; Hallgren and Pejler, 2006) and as angiogenesis and tumour progression modulators (Pos et al., 2005; Krauth et al., 2006). Other mast cell-derived proteins, as MMPs, interleukines and growth factors can also contribute to migration, proliferation and differentiation of surrounding cells *in vivo* (Sánchez-Jiménez et al., 2007). iii) It is obvious that mast cells also produce polyamines as essential compounds for macromolecular synthesis and cell survival. Consequently, they can be considered as a complex amine-producing- and amine-handling cell model.

Cross-effects of exogenous polyamines on mast cell physiology and those of exogenous histamine on tumour progression have also been reported by different authors (Medina et al., 2003; Theoharides and Conti 2004; Pos et al., 2005). Working with mouse C57 mast cells and rat basophilic leukemia cells, we observed that expression of both HDC and ODC were antagonic in the very early stages of the response to a prolonged and lethal phorbol ester plus dexamethasone treatment, suggesting an activation of histamine synthesis during the proliferative-resting transition. In the present work, we have checked this hypothesis working with human mast cells but replacing the antiproliferative stimulus to avoid colateral effects caused by exogenous compound additions. Thus, we have compared the expression patterns of HMC-1 cells in exponential phase of growth versus those of the same culture maintained in a serum-reduced medium (2.5% fetal calf-serum).

Figure 1 shows the growth profile of HMC-1 cells growing in culture medium at optimal (10%) serum concentration, but reduced up to 2.5% serum from day 5 on. As expected, after serum reduction, cell growth is stopped and cell viability starts to decrease. Samples from day 5 (just before serum reduction) and day 11 (6 days after serum-reduction) were analysed by using the amine-related probe macroarray.

Under any of the culture conditions described under Materials and methods, the transformed HMC-1 cell line expresses important quantities of GAPDH, β-actin, tryptase, HIF-1α, CDC-42, HDC, and c-myc, by decreasing the order of their signal intensities (up to 10% of the GAPDH intensity) (Fig. 2). The high levels of tryptase messenger were not a surprising result, as the protein can represent up to 25% of the intracellular protein content (90% of their secretory granules) (Sommerhoff et al., 2000). On the contrary, signals for both arginine and cysteine metabolism-related enzymes were barely detectable even after a 1-week exposure under any condition

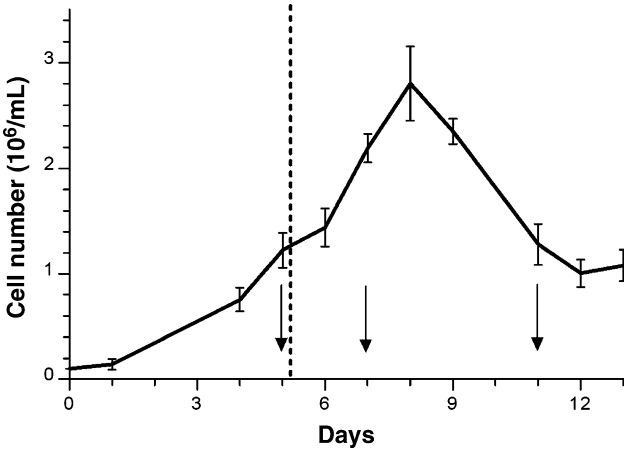

Fig. 1. Representative growth curve of the HMC-1 culture during the used serum-reduction protocol. The dash lines indicate the serum-reduction time. Arrows indicate the time for sample collections

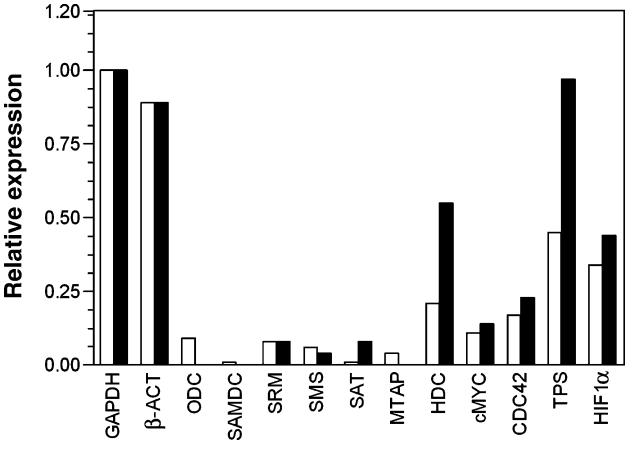

Fig. 2. Amine-related messenger movements between proliferating cells and non-proliferating HMC-1 cultures. Representative results of two macroarrays hybridized with samples from day 5 (white bars) and day 11 (black bars).The serum content of the medium has been reduced at day 5, as indicated in Materials and methods. Intensity of the GAPDH signal was considered as 1

assayed with this cell line, so far. On the contrary, the same alterations trends in the signal intensities of ODC, SAT and HDC were repetitively observed between proliferating (day 5) and serum-reduced cells (day 11). It was noteworthy that changes in HDC levels paralleled rather well with those observed for the tryptase (TPS) messenger. SAMDC, spermidine synthase (SRM) and spermine synthase (SMS) messengers were also detectable but no-clear changes between both experimental conditions could be deduced.

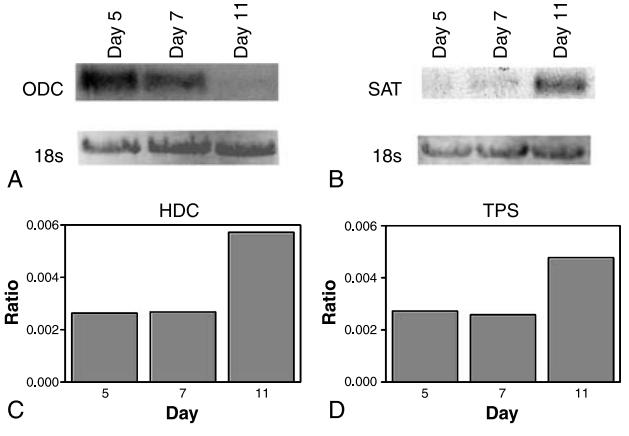

Fig. 3. Changes in ornithine decarboxylase (ODC), spermidine/spermine acetyl-transferase (SAT), histidine decarboxylase (HDC) and tryptase (TPS) mRNAs at different stages of HMC-1 serum-reduced cultures. **A** and **B** show representative Northern blots of ODC and SAT mRNAs, respectively, at day 5 (just before serum-reduction) and days 7 and 11 (2 and 6 days after serum-reduction, respectively). The negative images of the respective ethidium bromide-stained 18S rRNA bands are also shown for reference. **C** and **D** show representative results of HDC and TPS mRNAs measured by the real-time RT/PCR. HDC ratio was normalized against GAPDH as described by Melgarejo et al. (2006). TPS ratio was normalized againts beta-actin levels of the same samples, as described in Materials and methods

We have focused our attention on the changes of ODC, SAT, HDC and TPS to contrast with the macroarray results by alternative methods. Figure 3A shows representative Northern blot results for ODC and SAT messengers at days 5, 7 and 11. In comparison, ethidium-bromide 18S RNA levels corresponding to the same samples are also shown. ODC seems to be an early sensor of the antiproliferative signal since its levels were reduced just two days after the serum reduction and become almost undetectable at day 11. On the contrary, SAT shows undetectable levels in proliferating cells and increases its levels by several times in non-proliferating cells. These results coincide with those observed with the macroarrays even when using a different normalization reference. In addition, results obtained by quantitative RT/PCR methods also confirmed that serum deprivation not only induces an increase in HDC messenger levels (Fig. 3C), but also a parallel increase in TPS expression (Fig. 3D). This is an interesting result, since it adds further support to the existence of molecular mechanisms responsible for a coordinated synthesis of mast cell granule components, as suspected from the results obtained by different authors and rodent transgenic models (Forsberg et al., 1999; Ohtsu et al., 2001).

All these results together are in agreement with our previous observations on rodent basophilic models and support our hypothesis concerning an antagonic expression pattern between histamine and polyamine-related genes during cell proliferation/death transition in human mast cells. The molecular bases and the importance of this fact for inflammatory responses or tumor progression *in vivo* need further research and efforts. Nevertheless, they could indicate that histamine (and other mast cell mediator) synthesis could be regulated (in a coordinated manner) by stress signals. A direct relationship of polyamine reduction in the synthesis of these mediators cannot be assessed yet.

Finally, although human mast cells were chosen as the cellular model for validation of our first macroarray version, the array is continuously growing and could be applied to many other human cell types and physiopathological conditions.

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