

Mast cell transcriptome elucidation: what are the implications for allergic disease in the clinic and where do we go next?

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Mast cells (MCs) are principal effector cells of type-I-allergic reactions but still poorly defined in humans. The consortium Functional Annotation of the Mammalian Genome 5 has created a map of body-wide transcriptome data for a multitude of human cell types, including MCs. MCs were found to have a surprising transcriptional landscape expressing a range of genes not (or barely active) elsewhere in the body. Whereas several MC specific genes have no annotated function, others belong to networks defining specific MC traits and functions, such as granule architecture, IgER signaling, exocytosis and mediator production. Several of these genes are so highly enriched in MCs (versus all other cells), that they appear potentially specific targets for therapeutic interventions in diseases in which MCs are actively involved. We present some interesting candidates, highlight the uniqueness of MCs and discuss their role in allergy and itch sensation based on these renewed insights.

Mast cells (MCs) are firmly established as key effector cells of type-I allergic reactions, in which IgE bound to its high-affinity receptor (IgER, FcεRI) on the MC surface is cross-linked by a multivalent allergen to trigger a cascade of kinase-driven signaling events [1,2]. The subsequent release of both preformed and rapidly synthesized mediators causes the acute symptoms of allergic rhinoconjunctivitis, asthma, urticaria, food allergy and anaphylaxis. MCs have also been linked to a plethora of other disease conditions in mouse models. However, in the absence of human data, their direct transfer to the human situation is problematic due to profound differences between human and rodent MCs [3]. So, what do we really know about these cells in humans? Recent publications by functional annotation of the mammalian genome 5 (FANTOM5), a large consortium dedicated to body-wide transcriptomics and the identification of genomic

regions which actually control gene activity revealed that MCs may have properties not associated with the cells before, but conversely lack functions of other immune cells commonly linked to MCs [4,5]. From an allergy point of view, these data not only offer novel insights into how the allergy machinery works at the level of MCs but they also indicate potential novel drug targets for the alleviation of allergy. This editorial shortly summarizes this recent progress made for MCs of human skin origin.

The FANTOM5 project

In the FANTOM5 project, the cap analysis of gene expression coupled to high-throughput next-generation sequencing at the single molecule level was applied to a collection of nearly 1000 samples, mapping transcription start sites and quantifying relative gene expression levels in a genome-wide fashion [4]. Thus, the one major advantage of the resulting

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expression atlas is that the data obtained for one cellular state can be put into a global context by direct comparison with other primary cells, cell lines or tissues. Another improvement specifically regarding the MC samples is that these cells were characterized *ex vivo* and compared to counterparts that were likewise derived from skin, but subjected to expansion in culture. Substantial differences between the two subsets underline a remarkable adaptability of MCs to altered micromilieus. However, they also emphasize that results based on cultured MCs should be viewed with some caution, since tissue-deprived MCs may not accurately reflect their *in situ* counterparts, and this can have implications for therapeutic considerations. In fact, we find various genes that are induced in MCs upon culture, but missed in the *ex vivo* samples, while the activity of an even greater number of genes disappears outside of the tissue. This is highlighted by the following numbers: overall, 33,515 annotated and other expressed regions were found to be active in MCs, with 5826 being differentially expressed between *ex vivo* and cultured MCs; of these, a total of 85.5% were downregulated in culture.

MCs are unique

FANTOM5 uncovered a unique and surprising transcriptional landscape of MCs, characterized by several 'private genes' strongly expressed by MCs but weakly or not expressed by other constituents of the human body, combined with general hematopoietic genes, but specific absence of other immune-related genes (SUPPLEMENTARY TABLE 1 [supplementary material can be found online at www.informahealthcare.com/suppl/1744666X.2014.933074] contrasts MCs against other myelocytes). In addition, MCs express multiple genes not associated with the lineage before, some of them important genes of unrelated tissues, yet others with unknown function. Clearly, unraveling their role in MCs will improve our understanding of human MC biology. Befitting their uniqueness, MCs have no close relative in the hematopoietic network, the best concordance found with hematopoietic progenitor cells. The overall concordance with basophils, the other major effector cell of allergic reactions, is fairly low [5], and the same applies to other myelocytes (SUPPLEMENTARY TABLE 1).

MCs & allergy

Not surprising, skin MCs were confirmed to express high levels of the three chains that make up the high-affinity IgER (α , β , γ) [6], in addition to the genes related to the production of granule-associated mediators (including histamine, tryptases, chymase), which become exteriorized in allergic reactions. Heparin is essential for granule architecture and mediator storage of MCs [7,8]; accordingly, enzymes involved in the biosynthesis of heparin were likewise enriched in MCs, including NDST2 [7,8]. Befitting the vast accumulation of histamine, MCs expressed VMAT2 at highest levels (SLC18A2), which transports histamine into granules (SUPPLEMENTARY TABLE 1) [9].

MCs do not only degranulate after IgER aggregation, but they also synthesize lipid mediators and activate various genes. In FANTOM5, MCs were verified to express highest levels of

the enzymes responsible for PGD2 and LTC4 generation (HPGDS, LTC4S), while the *de novo* induced genes comprised chemokines, cytokines and receptors involved in cell-cell communication, confirming previous profiling results [10,11]. The upregulated genes substantiate a crucial role of MCs in the recruitment of different types of CCR-expressing immune cells into the tissue upon IgER-mediated activation, potentially followed by physical interaction with these cells.

With respect to MC degranulation, several players could be identified as highly MC expressed. As an example, members of the Rab family of small GTP-binding proteins are highlighted here, for example, RAB27B and RAB37, both implicated at the distal stages of exocytosis by organizing vesicle trafficking [12,13]. Especially, RAB27B was enriched in MCs, while much lower levels were found in basophils and other myelocytes (SUPPLEMENTARY TABLE 1).

MCs & itch

In the skin, MCs are spatially close to C neuron terminals, and the MC-neuronal cross-talk is critically implicated in the pathophysiology of itch [14]. A variety of MC mediators, especially histamine and tryptase, are important factors of pruritis by the activation of histamine receptors (H3R, H4R) and PAR2, respectively. These were confirmed in FANTOM5 to be exclusively (tryptases) or predominantly (HDC) expressed by MCs. H4R antagonists (e.g., JNJ-7777120) have been demonstrated to be efficacious in models of pruritus, and clinical trials are ongoing [14]. We uncovered that H4R, expressed by skin MCs [15], is unexpectedly highest in these cells across the atlas. This raises the interesting possibility that MCs are important target cells of the histamine they release, that this occurs predominantly via H4R and that the efficacy of H4R antagonists in the allergic context also stems from *direct* actions on MCs.

IL-31 is another critical player in the pruritis network [14]. In FANTOM5, IL-31 expression seemed exceptional, because it was only expressed in one of the stimulated MC samples. When testing MCs from various individuals for IL-31 production, we confirmed highly variable levels, substantiating that the propensity of MCs to produce this mediator differs among humans. The variable tendency of individuals to produce this cytokine may contribute to diseases characterized by chronic itch [16,17]. Thus, targeting IL-31 in selected patients may become one approach of personalized medicine in the context of pruritic disorders.

Genes with inhibitory function

As mentioned, the greatest attraction of the FANTOM5 datasets is the simultaneous availability of transcriptome data for a body-wide collection of samples. Among the genes overexpressed by MCs, cell surface receptors comprised a significant subgroup. Several of these have well-defined roles in the activation of distinct pathways or in the transduction of inhibitory signals, which have co-evolved to dampen stimulatory signals in order to decrease harmful effects of prolonged activation. It is the latter category, on which we focus here.

While several inhibitory receptors described for MCs were in general not (particularly) MC specific (SIRPA, PILRB, LAIR1, CD72, CD84, CD300A), CD200R displayed highest expression in MCs, though it was not differential in MCs versus myelocytes (no appearance in SUPPLEMENTARY TABLE 1). Two members of the SIGLEC family, which also deliver negative signals by tyrosine-based inhibitory motifs, seemed more interesting. In fact, SIGLEC6 and SIGLEC8 were highly enriched in MCs, with SIGLEC6 being 200–400-fold overexpressed in MCs versus the mean of non-MC FANTOM5 samples (position 17 in SUPPLEMENTARY TABLE 1). While SIGLEC8 has been discovered as a drugable target of MCs [18], SIGLEC6 has been rather investigated in placenta [19]. Our data indicate that it may well be worth further investigation especially in MCs.

When discussing counter-balancing signal transduction, RGS13 deserves special mentioning, as its expression was approximately 500-fold higher in MCs versus the mean of non-MCs, qualifying it as a novel lineage marker (position 8 in SUPPLEMENTARY TABLE 1). RGS13 is a regulator of G-protein signaling, involved in the fine-tuning of various cellular events. RGS13 has well-understood functions in MCs where it operates as a negative regulator of IgE-mediated and G-protein triggered MC responses [20]. With the knowledge that RGS13 is confined to MCs, augmenting its activity may afford an excellent strategy in MC-dependent diseases, in which aberrant anaphylactic or non-immunologic MC activation takes place.

Conclusion

FANTOM5 further demystified human MCs and revealed their unique character. The datasets offer the excellent opportunity not only to quantitate genome-wide transcript levels in MCs, but also to deduce the degree of specificity for the MC

lineage. *A priori*, the best therapeutic candidates would be the ones highly expressed by MCs but not or weakly expressed by non-MCs. In fact, MCs display various genes that fulfill these criteria, several of which with ascribed inhibitory functions. If developed into pharmaceuticals, antibodies or small-molecule agonists would potentially offer new targeted therapies in disease states where these cells are important without collateral tissue damage or side effects outside of the MC system.

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