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Review

Molecular basis of mast cell disease☆

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ABSTRACT

Mastocytosis is an incurable and sometimes fatal haematological disorder grossly described as the accumulation of abnormal mast cells in the bone marrow and other organs causing tissue and organ damage. The clinical manifestations of this disease are extremely variable; disease phenotypes range from indolent to aggressive, and often present with associated non-mast cell haematological disorders (AHNMD), mainly myeloproliferative neoplasm and myelodysplastic syndromes. Recent efforts to genetically dissect the mechanisms that define aggressive and non-aggressive mastocytosis have generated a list of recurrent somatic mutations in mastocytosis patients that are associated with and may predict the evolution towards aggressive disease phenotypes. Here we review these mutations and discuss the molecular mechanisms associated with these mutations in an effort to better understand the biology of this disease and to predict its onset and evolution, with the ultimate goal of devising new and improved treatment strategies.

1. Mastocytosis

Mastocytosis is a rare and heterogeneous disease characterized by the accumulation of morphologically and phenotypically abnormal mast cells in one or several organs causing tissue damage and, in more aggressive cases, organ failure. Systemic mastocytosis (SM) disease phenotypes range from indolent to aggressive and are defined by WHO criteria: mainly B- and C-findings that describe the extent of organ and tissue damage resulting from systemic mast cell infiltration (Pardanani, 2012; Sánchez-Muñoz et al., 2011; Sperr and Valent, 2012; Valent et al., 2001). Clinical presentation of mastocytosis is extremely variable and other symptoms can, but do not necessarily include elevated serum tryptase levels, flushing, cutaneous skin lesions, muscle pain, osteoporosis and diarrhoea. Mastocytosis occurs in both children and in adults where the former has a tendency to spontaneously regress and the latter is an incurable clonal disease prone to chronic and more severe symptoms. In about 40 percent of cases, systemic mastocytosis is diagnosed in conjunction with associated clonal haematological non-mast cell lineage diseases, AHNMD, pre-dominantly myeloproliferative and myelodysplastic syndromes (Lim et al., 2009; Wang et al., 2013).

Where all forms of mastocytosis are associated with activating mutations in *KIT*, a receptor tyrosine kinase and a key regulator of mast cell biology, currently there are no biomarkers to predict either the onset or the evolution of disease (Donker et al., 2008). In a majority of cases, patients with cutaneous mastocytosis (CM) or indolent disease with mild or intermittent symptoms are treated conservatively using prophylactic anti-mediator drugs to counter symptoms. Aggressive treatment options can include cytoreductive therapy using IFN-á or 2CdA (Sperr and Valent, 2012). Receptor tyrosine kinases are also validated therapeutic targets, but resistance to tyrosine kinase inhibitors (TKIs) is frequent and for many can manage disease but not cure. Moreover, about 80% of mastocytosis cases present with the KITD816V mutation. This form of the receptor is inherently resistant to most common TKIs (Ma et al., 2002). For example, a majority of mastocytosis patients are refractory to Imatinib therapy and patients that have other *KIT* mutations or wild-type *KIT*, only sometimes respond (Zermati et al., 2003).

Additional inhibitors aimed at RTK-independent pro-oncogenic pathways could allow for therapeutic combinations and reduced doses of drugs to limit adverse effects and the risk of drug resistance. While a role for oncogenic *KIT* in driving mast cell disease is clear, the mechanisms driving the multiple phenotypic and clinical manifestations of this disorder are not known. It is likely that,

Abbreviations: SM, systemic mastocytosis; AHNMD, associated clonal haematological non-mast cell lineage disease; CM, cutaneous mastocytosis; RTK, receptor tyrosine kinase; MDS, myelodysplastic syndrome; MPN, myeloproliferative syndrome; AML, acute myeloid leukaemia.

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like in other haematological diseases, additional *KIT*-independent signalling molecules and pathways play a more important, or even decisive, role in growth of neoplastic cells. Taken together, understanding the role of KITD816V and cooperating mutations in aggressive forms of mastocytosis could provide a paradigm for the discovery of drugable targets and novel treatment strategies to improve response and outcome in TKI-resistant patients.

While the majority of patients present with indolent, cutaneous forms of mastocytosis, about 15% of patients present or develop aggressive forms of systemic mastocytosis with various phenotypes including mast cell leukaemia and sarcoma and often accompanied by AHNMD. At present it is unknown why these patients progress while others do not. Moreover, it is debated whether AHNMDs are part of the disease or separate entities that should be treated in parallel. Still less is understood concerning the molecular mechanism of spontaneous regression of childhood mastocytosis, although there is evidence to suggest that distinct *KIT* mutations may be biologically important in changing the course of disease.

Because *KIT* mutation is consistently found in both aggressive and non-aggressive SM, one hypothesis is that the accumulation of additional mutations is the primary cause of aggressive disease evolution. The recent sequencing of a large panel of genes for mutations previously identified in other myeloid malignancies has provided a list of recurrent somatic mutations in mastocytosis patients, including novel *KIT* mutations, mutations in epigenetic factors, splicing factors and other signalling molecules (Table 1). By far the most frequent mutations identified to date are *KIT*, *TET2* and *SRSF2*. Other mutations frequently found in MDS/MPN/AML are less frequent or absent in SM patients (e.g. *IDH1*, *DNMT3a*, *EZH2*, *TP53*). The goal of this review is to describe the molecular and biological consequences of these mutations and to discuss how mutations that frequently occur together in patients could cooperate to explain certain aspects of mast cell disease and prognosis that have so far remained elusive.

2. Common mutations in mastocytosis

2.1. KIT

KIT is a type III transmembrane receptor tyrosine kinase (RTK). In the hematopoietic system, KIT is specifically expressed in stem, early progenitor, and mature mast cells where is an important determinant of both cell fate and phenotype (Valent, 1994; Krishnaswamy et al., 2006). The clonal proliferation of mast cells associated with disease is, in the overwhelming majority of cases, secondary to a gain-of-function of *KIT* that results in constitutive signalling through this receptor in the absence of its cognate ligand stem cell factor (SCF) (Furitsu et al., 1993; Casteran et al., 2003). Indeed, transgenic mouse models have confirmed a role for KIT mutation in driving mast cell disease when activated in either progenitor or differentiated mast cells in the hematopoietic system (Gerbaulet et al., 2011; Zappulla et al., 2005).

Systematic sequencing of samples from patients suffering from mastocytosis has revealed that adults are mainly mutated on tyrosine kinase domain of *KIT* (TKD-KITD816V), while half of children harbour mutations in the extracellular domain (ECD-KITdel417-419insY, KITK509I and KIT-ITD502-503) (Bodemer et al., 2010). Interestingly, where both TKD- and ECD-KIT mutants display gain-of-function activity, they provoke different cellular phenotypes, gene expression profiles and signal transduction (Yang et al., 2010). Common gain-of-function activities of both TDK-KIT and ECD-KIT include auto-phosphorylation of the receptor kinase domain in absence of ligand, constitutive activation of downstream signalling, induction of cytokine-independent proliferation and increased survival of mast cells in the absence of growth factors. At a molecular level, however, the TKD-KIT mutant shows decreased Akt signalling and microarray analysis shows that ECD-KIT and TKD-KIT provoke distinct transcription profiles when expressed in human cell lines (Yang et al., 2010) or in primary murine mast cells (Soucie E, unpublished results). More specifically, the TKD-KIT mutant induces a greater number of genes associated with mast cell differentiation (Yang et al., 2010).

Interestingly, although very few patients have been studied at a molecular level so far, mast cell leukaemia (MCL) in adults appears to be associated with mutations located at or near amino acid positions 502–503 of *KIT* (Georgin-Lavialle et al., 2012, 2013a), more often found in GIST patients (Emile et al., 2012; Lasota and Miettinen, 2006). The biological consequences of KIT-ITD502-503 are less studied but the presence of this distinct mutation suggests a separate aetiology for MCL. The advent of transgenic mice carrying this allele should provide insight into the consequences of this mutation for both hematopoietic progenitors and mast cells.

Mast cell sarcoma (MCS), an always-fatal disease found in both adults and children, is another rare entity with only ten cases reported in the literature to date. MCS is associated with an absence of *KIT* mutation, although in 2 patients novel *KIT* mutations were found: KITL799F (Kim et al., 2013) and KITV560G (Georgin-Lavialle et al., 2013b). The significance of these mutations and their role in disease is not known.

2.2. Epigenetic regulators

Mutations in genes that express factors implicated in epigenetic regulation, including histone and DNA modifying enzymes, have been implicated in numerous diseases including many haematological disorders (Shih et al., 2012; Petronis, 2010). After *KIT*, the second gene found most frequently mutated in SM is *TET2*, an enzyme involved in the active demethylation of DNA. An important number of patients with SM also harbour mutations in other epigenetic regulators including *ASXL1* and *IDH2*.

2.2.1. TET2

TET proteins, TET1 TET2 and TET3, are enzymes that catalyze the conversion of 5-methyl-cytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and play an important role in the active de-methylation of DNA (Petronis, 2010). DNA de-methylation induces a more open chromatin conformation, displaces methyl CpG-binding domain proteins (MBDs) and is generally correlated with active histone marks (Hughes et al., 2013). Mutations in the *TET2* gene are frequent in myeloproliferative syndromes and up to 20 percent of mastocytosis patients harbour at least one *TET2* mutation (Table 1). *TET2* mutation is also correlated to aggressive disease in systemic mastocytosis patients (Soucie et al., 2012).

TET2 is expressed in various tissues, but its expression appears to be highest in immature or progenitor cells (Langemeijer et al., 2009). Mutations in *TET2* result in a loss of function of the TET2 protein, and generally, are associated with a DNA hypermethylation phenotype (Ko et al., 2010). Clonal and sorted cell population analysis has revealed that somatic mutation of *TET2* occurs in early progenitor cells and likely pre-disposes rather than drives cellular transformation. This is supported by the hematopoietic phenotype of *TET2* knockout mice that have a defect in the homeostatic control of the stem cell/progenitor cell compartment and develop spontaneous neoplasm only at a late age (Moran-Crusio et al., 2011; Quivoron et al., 2011).

DNA methylation is one of the best-characterised epigenetic modifications and the CpG-island hypermethylated phenotype (CIMP) has been correlated to cellular transformation and disease (Hughes et al., 2013). Studies in both embryonic and hematopoietic stem cells have revealed an important role for DNA methylation during development and distinct cell lineages have distinct DNA

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