Christin Maria Duffert
Dr. med.

Different Molecular Features Suggest Distinct Disease-causing Mechanisms in C-Mpl Gain-of-function Mutations

Fach: Kinderheilkunde
Doktorvater: Herr Prof. Dr. med. Andreas Kulozik, PhD

Interaction between the c-Mpl ligand THPO and its receptor is crucial for the regulation of thrombopoiesis. The dynamics of this process are subject to alterations within the gain-of-function mutants c-Mpl-P106L, c-Mpl-S505N, c-Mpl-W515K and c-Mpl-W515L, which are all associated with chronic thrombocytosis. Apart from this common feature, heterogeneous and possibly severe complications may occur in affected individuals. Thus, an increased risk of thrombotic events and myelofibrosis was observed in clinical studies with patients harboring the c-Mpl-S505N mutation. Furthermore, occurrence of c-Mpl-W515K and c-Mpl-W515L is associated with myeloproliferative neoplasms and cases of leukemic transformation in their presence were reported. However, so far no such clinical complications have been described in individuals displaying c-Mpl-P106L. In addition to the diverse clinical symptoms, different circumstances of occurrence were observed for the named mutations. The germline mutation c-Mpl-P106L is transmitted in an autosomal-recessive mode of inheritance, whereas autosomal-dominant transmission as germline mutation in the great majority of cases and sporadic occurrence as somatic mutation were described for c-Mpl-S505N. By contrast, both c-Mpl-W515K and c-Mpl-W515L have so far only been reported to occur as somatic mutations. To date, the striking differences in clinical manifestation across these c-Mpl gain-of-function mutations are insufficiently understood. Better insight into the disease-causing mechanisms is needed in order to define the respective health risks precisely, determine optimal surveillance and treatment strategies and achieve the best possible health outcomes for affected patients.

Starting from the clinical observations laid out above, the present study examined the hypothesis that different molecular mechanisms account for transmission of the disease-causing effect across the c-Mpl-gain-of-function mutants c-Mpl-P106L, c-Mpl-S505N, c-Mpl-W515K and c-Mpl-W515L. In order to gain more insight into these molecular processes, the present work investigated and compared key functional properties of these mutants by means of a cell culture model. For the better-explored c-Mpl-P106L mutation, impaired receptor glycosylation, abnormal subcellular distribution and an absence of surface expression were previously described as striking molecular hallmarks. In presence of this mutation, the lack of receptor surface expression furthermore results in abnormally elevated THPO serum levels as sufficient internalization and degradation of the hormone cannot be realized. This disruption of the regular THPO negative feedback-loop is likely to play an essential role within the disease-causing mechanism of c-Mpl-P106L.

The present study therefore examined, whether and to which degree the striking molecular characteristics of c-Mpl-P106L apply to the functionally less-explored gain-of-function mutants c-Mpl-S505N, c-Mpl-W515K and c-Mpl-W515L. Hence, three crucial stages of the THPO-receptor life cycle were addressed: (1) post-translational processing of the immature receptor protein and its concomitant subcellular distribution, (2) cell surface expression of the mature receptor and (3) receptor internalization upon stimulation with its ligand, THPO.

The experimental results clearly demonstrated major differences in the molecular properties of the c-Mpl mutants and therefore suggest the existence of different disease-causing mechanisms across the

gain-of-function mutants in question. First of all, the present findings highlighted that both impaired and regular receptor glycosylation occur in the examined c-Mpl gain-of-function mutants, which is reflected by differential subcellular receptor distribution. Thus, in contrast to previous observations regarding c-Mpl-P106L, the results indicated regular processing and concomitant subcellular distribution of receptor proteins for the mutants c-Mpl-S505N, c-Mpl-W515K and c-Mpl-W515L, respectively. Second, the results demonstrated that the examined c-Mpl gain-of-function mutants differ regarding surface expression levels. Thus, as the most striking molecular difference compared to c-Mpl-P106L, the mutants c-Mpl-S505N, c-Mpl-W515K and c-Mpl-W515L all displayed stable surface expression of receptor proteins. In the present results, c-Mpl-W515K and c-Mpl-W515L surface expression levels were comparable to those of c-Mpl-wildtype, whereas c-Mpl-S505N showed surface levels inferior to the wildtype. Third, on top of variations in receptor glycosylation, subcellular distribution and surface expression patterns, the present experimental results hinted at differences in the maintenance of the THPO negative feedback-loop across c-Mpl gain-of-function mutants. Indeed, in contrast to c-Mpl-P106L, it seems likely that the THPO negative feedback-loop is preserved in c-Mpl-S505N, c-Mpl-W515K and c-Mpl-W515L. Therefore, it may be hypothesized that a maintained THPO negative feedback-loop is sufficient to prevent dysregulation of THPO serum levels but not transmission of the c-Mpl gain-of-function effect resulting in thrombocytosis.

Taken together, the experimental results of the present study allowed a classification of c-Mpl gain-of-function mutants based not only on their observed clinical correlates but factoring in their molecular properties. This constitutes an important extension to classifications found in the current literature as for example the recently suggested categories by He *et al.*, which are primarily based on clinical features. As the most striking point, the present results immediately suggest a classification of the c-Mpl-P106L mutation in a separate category. Based on the mutation's distinct molecular features, this categorization would constitute an essential extension to the current classification proposed by He *et al*. Regarding the separate categories of c-Mpl-S505N on the one hand and c-Mpl-W515K/L on the other hand, the current classification seems appropriate due to the mutations' deviating clinical contexts. Future research may further elucidate the respective molecular mechanisms behind their different clinical correlates. As one promising approach, investigation of the mutations' respective pathway activation patterns may add to further clarification of their disease-causing effects. In the future, better understanding of these molecular mechanisms shall allow researchers to develop optimal treatment strategies for affected patients.

In summary, the present study detected major molecular differences across the c-Mpl gain-of-function mutations c-Mpl-P106L, c-Mpl-S505N, c-Mpl-W515K and c-Mpl-W515L. These findings suggest the existence of different disease-causing mechanisms behind the mutations' respective clinical correlates and provide the basis for an important extension to the current classification of c-Mpl mutations.