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The -2518 A/G single nucleotide polymorphism of MCP-1 in myelofibrosis: functional characterization on ex-vivo patient cells

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Host genetic variations have an essential role in the mutational landscape of Philadelphia-negative MPN [1,2]. However, the contribution of inherited factors in disease phenotype and evolution is poorly characterized. In MPNs, chronic inflammation triggers neoplastic transformation and catalyzes clonal evolution toward end-stage disease [3]. We recently demonstrated that the -2518 A/G SNP of the Monocyte Chemoattractant Protein-1 (MCP-1, rs1024611) is an inherited host genetic factor associated with secondary myelofibrosis (sMF) and a biomarker of disease severity in MF [4]. Here we aimed to characterize MCP-1 expression in MF according to patients' genotype, and the potential cellular source(s) of this chemokine. For this study, 15 therapy-naïve MF patients were recruited, 4 healthy subjects and 4 apheresis bags were utilized as controls (CTRL). MF were stratified according to their rs1024611 genotype in A/A (wild type), A/G and G/G (polymorphic). Peripheral blood mononuclear cells (MNCs) were isolated by Ficoll-Hypaque gradient, in part pelleted (resting, T0) and in part seeded in RPMI-1640 medium with 1.1 ng/ml of IL-1 β for 20 hrs (T1). T0 and T1 cells were processed for RNA extraction. $CD34^+$-cells were purified from MF peripheral blood and from apheresis bags by immunomagnetic selection and differentiated toward the MK lineage as previously described [5]. Mature $CD41^+$ MKs were then processed for RNA extraction. MCP-1 expression was evaluated by real-time PCR. We demonstrated that MF-MNCs significantly over-expressed MCP-1 as compared to CTRL-MNCs at basal state. Upon IL1β stimulation, we observed a dose-dependent effect of the -2518 A/G SNP on MCP-1 expression, with polymorphic patients displaying a >100-times higher fold-increase (T1 vs. T0) in MCP-1 levels as compared to A/A. MF-MKs also showed a significantly higher expression of MCP-1 as compared to CTRL. Finally, MF-$CD34^+$-cells from A/G+G/G patients displayed impaired MK differentiation compared to A/A, as indicated by a significantly lower number of $CD41^+$-cells obtained in culture. Our data show that circulating MNCs and $CD34^+$-derived MKs are a major source of MCP-1 in MF. Polymorphic MF patients, who cluster with adverse hematologic characteristics, display here a higher capacity to over-express MCP-1 under an inflammatory stimulus and an impaired megakaryocytic differentiation potential. Further studies to better define the role of MCP-1 on $CD34^+$-cells differentiation in the context of MF are desirable.

References

[1] Trifa et al. (2018) MECOM, HBS1L-MYB, THRB-RARB, JAK2, and TERT polymorphisms defining the genetic predisposition to myeloproliferative neoplasms: A study on 939 patients. Am J Hematol. 93(1): 100-106
[2] Tapper et al. (2015) Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. Nat Commun. 7;6: 6691
[3] Hasselbalch (2012) Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer? Blood. 119(14): 3219-25
[4] Masselli E et al. (2018) The -2518 A/G polymorphism of the monocyte chemoattractant protein-1 as a candidate genetic predisposition factor for secondary myelofibrosis and biomarker of disease severity. Leukemia. doi: 10.1038/s41375-018-0088-y
[5] Masselli E et al. (2015) Protein kinase Cvarepsilon inhibition restores megakaryocytic differentiation of hematopoietic progenitors from primary myelofibrosis patients. Leukemia. 29: 2192 – 201

Key words

Myelofibrosis, MCP1, -2518 A/G SNP.