ALTERNATIVE SPLICING VARIANT COULD BE RESPONSIBLE OF MORGANA UNDER-EXPRESSION IN ACML

C. Panuzzo[1],* G. Carra[1], S. Crivellaro[2], R. Piazza[3], U. Familiari[2], S. Rocca[4], F. Fusella[4], G. Saglio[2], M. Brancaccio[4], A. Morotti[2]
[1]Clinical and Biological Sciences, [2]San Luigi Hospital, Turin, [3]Dep. of Health and Sciences, Monza, [4]University of Turin, Turin, Italy

Background: Morgana/chp-1 is a chaperone protein regulating ROCK activity and it is involved in centrosome duplication and tumorigenesis. Morgana +/- mice develop with age a fatal transplantable myeloproliferative disease similar to aCML, presenting centrosome amplification as well as cytogenetic abnormalities in bone marrow (BM). Notably, low morgana expression levels were found in the BM of aCML-affected patients and in a portion of CML Philadelphia positive patients. To assess whether morgana gene (CHORDC1) mutations can be responsible for low morgana expression levels in aCML patients we screened exome sequencing data obtained; no mutation were detectable suggesting that more complex regulatory mechanisms are involved in Morgana underexpression.

Aims: The aim of this study was to understand the mechanism responsible of Morgana reducted expression in aCML in order to identify a new pathway druggable to restore Morgana levels.

Methods: RNAs extracted from 9 primary aCML bone marrow samples collected at diagnosis after informed consent was reverse transcripted in c-DNA using random examers. The amplification products derived from Morgana gene were purified using Gel Extraction System and sequenced with ABI Prism 3100 Capillary Genetic Analyzer (Applied Biosystems). To evaluate the amount of the wild type and alternative transcript, we used two specific TAQMAN probes, one able to recognise only the wild type form and one other able to recognise both the transcripts. Protein analysis were performed by immunohistochemistry (IHC) experiments on formalin-fixed, paraffinembedded bone marrows. Exome sequencing was used to extend to a larger portion of aCML patients and to analyze another pathology (AML) in order to verify the specificity of this inactivation.

Results: In addition to the main Morgana mRNA we found the co-existence of another transcript; direct sequencing of amplification products confirmed the presence of an alternative transcript (AS) which lacks the entire exon 3 of Morgana; this phenomenon produces a change on the correct reading frame and as consequence after 9 nucleotide the generation an earlier STOP codon. The product of this new transcript isn’ t a functional protein. Next we decide to quantify this alternative transcript by using one probe able to recognize both the transcripts and one probe spanning the exons 4 and 5 of Morgana and so able to recognize only the wild type form. We observed that unlike the group of healthy donors all the aCML where Morgana wild type is low have an high presence of alternative transcript. Surprisingly in the little percentage of aCML with normal Morgana level the alternative splicing is not significantly expressed. The generation of this AS colud be the mechanism

responsible of Morgana down-expression in aCML; in fact all the patients with high alternative trascript shown very low level in Morgana protein, analysed by IHC. All these data where confirmed by exome sequencing, where the alternative transcript is observed on a large portion of aCML while in 10 AML tested it is not detectable, suggesting the specificity of this type of regulation in this myeloproliferative neoplasm

Summary and Conclusions: In this preliminary work, we identify one Morgana AS responsible of aCML reduced Morgana expression. This could be a consequence of mutations of those genes that are involved in the regulation of the spliceosome even in the absence of spliceosome gene mutations, alternative splicing of specific genes has also been reported with important biological consequences and potential therapeutic opportunity.