Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2021.37.005944

SF3B1 Gene Abnormalities are Not Common in Peripheral T-Cell Lymphomas-Not Otherwise Specified

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ARTICLE INFO

Received: June 25, 2021

Published: July 08, 2021

Citation: Maryam Etebari, Mohsen Navari, Davide Gibellini, Serah Kaggia, Pier Paolo Piccaluga, et al., SF3B1 Gene Abnormalities are Not Common in Peripheral T-Cell Lymphomas-Not Otherwise Specified. Biomed J Sci & Tech Res 37(1)-2021. BJSTR. MS.ID.005944.

ABSTRACT

The molecular basis of Peripheral T cell lymphoma not otherwise specified (PTCL/NOS) has remained essentially elusive, due to heterogeneous nature of this malignancy. Recently recurrent genetic lesions affecting normal RNA splicing have been described in different hematological malignancies. Therefore, this study aimed to investigate the possible role of SF3B1 gene, the core component of splicing machinery, in PTCL/NOS pathogenesis.

Keywords: Peripheral T-Cell Lymphoma not Otherwise Specified; Next Generation Sequencing; SF3B1; RNA-Sequencing; Whole Exome Sequencing; Sanger Sequencing

Introduction

Peripheral T-cell lymphoma not otherwise specified (PTCL/NOS) represents the largest and the most heterogeneous group of peripheral T cell lymphomas with extremely variable pathological and molecular features. Until now, the molecular pathology of this tumor is poorly understood [1,2]. Nonetheless, gene expression profile (GEP) studies indicated consistent abnormalities in selected pathways [3-9]. More recently, next generation sequencing (NGS) studies revealed some of the molecular bases sustaining the transcriptional abnormalities. Particularly, the newly recognized category of follicular T-helper (TFH) related PTCLs, including angioimmunoblastic lymphoma (AITL), follicular T-cell lymphomas (FTCL), and some PTCLs/NOS with TFH phenotype showed a consistent genetic landscape, characterized by somatic mutations affecting RHOA, TET2, IDH2, and DNMT3A [10-12]. For the remaining PTCL/NOS cases, the genetic pattern appeared quite

heterogeneous, with a few recurrent mutations affecting the T-cell receptor signaling, the JAK/STAT pathway, and the epigenetic controlling machinery [13].

In the last decade, however, beside gene expression patterns and somatic mutations occurrence, NGS technology has also provided extensive information about different genetic events such as, chromosomal translocations, insertions, deletions, and, remarkably, abnormal mRNA splicing. Abnormal mRNA splicing may result from mutations of splice site sequences, mutations in splicing regulatory sequences, and mutations in genes that contribute to constitute the so-called splicing machinery or spliceosome [14]. It is now becoming apparent that somatic mutations of spliceosome genes can play a role in the pathogenesis of human cancers, in particular in the pathophysiology of hematologic malignancies (both myeloid and lymphoid) as well as in solid tumors [14-23]. The spliceosome is a large RNA-protein complex, composed of five small nuclear RNAs (snRNAs) associated with proteins to form particles termed small nuclear ribonucleoproteins (snRNPs). The Splicing Factor 3b Subunit 1 (SF3B1) protein functions at the catalytic core of the spliceosome [17-19]. Recently, whole exome sequencing (WES) studies uncovered frequent somatic mutations in splicing machinery components, especially SF3B1, in patients with myelodysplastic syndrome (MDS) and these mutations are particularly common (up to 80% of cases) in those cases associated with increased sideroblasts [14-18]. Similarly, in chronic lymphocytic leukemia (CLL), SF3B1 was found to be the second most frequently mutated gene [24-29]. SF3B1 mutations were also detected at lower frequency in a variety of solid tumors such as gastric, prostate, breast, and renal cancers as well as others [14,30].

It is still unclear, however, the functional role SF3B1 mutations in carcinogenesis, and it has not been well established whether deregulated SF3B1 activity is required for the maintenance of cancer [30]. It is currently believed that SF3B1 mutations might affect multiple cellar functions and pathways, including DNA-damage response, heme biosynthesis, R-loop formation, and telomere maintenance [30], as well as Notch and NF-κB pathways [30]. This study aimed to investigate the possible presence of SF3B1 gene abnormalities in PTCL/NOS.

Materials and Methods

We collected formalin fixed paraffin embedded blocks (FFPE) from 41 individuals with PTCL/NOS. The cases were diagnosed as PTCL/NOS according to WHO classification criteria at Sant'Orsola Malpighi Hospital, Bologna, Italy [1,2]. Tumor cell percentage was higher than 70% in all examined cases based on morphological and immunophenotypical analyses. The sample size (N≥30) was calculated in order to have more than 95% of probability to detect a mutation recurrent in 10% of cases. Genomic DNA was extracted from all samples using QIAamp DNA mini extraction kit according to the manufacturer's protocol (QIAGEN, Italy). Following, polymerase Chain Reaction (PCR) was performed to amplify the exons no. 14, to 16 of SF3B1 gene which are reported as mutational hotspots [14]. Primers and relative conditions for amplifying were described by Rossi et al. [27]. The PCR products were purified using MinElute PCR Purification Kit (QIAGEN) and were sequenced with the original PCR primers using the BigDye Terminator v1.1 Cycle Sequencing Kit and a Genetic Analyzer (Applied Biosystems).

Results and Discussion

All sequences were then manually examined and revealed no mutation in the studied exons of SF3B1. To extend our experience and to make our data more robust we additionally studied NGS data obtained by WES of 10 cryo-preserved PTCL/NOS cases, matched with non-neoplastic DNA as well as RNA-sequencing of 23 PTCL/NOS cases (manuscript in preparation). All these data had been obtained by Illumina technology (for both library preparation and sequencing) (Illumina, CA). Interestingly, RNA-sequencing revealed a high frequency of splicing variant, not encountered in normal lymphocytes. However, again, consistent with Sanger sequencing results, bioinformatic analysis of NGS data [31,32] revealed no abnormality in any exons of SF3B1. As it has been mentioned, in this study we focused on SF3B1 gene since it had been shown to play a central role in the pathogenesis of hematologic tumors, and in a variety of solid tumors. However, various reasons could be accounted for generation of abnormal mRNA splicing, such as mutations in genes of splicing machinery, mutations of splice site sequences, and mutations in splicing regulatory sequences. Mutations affecting MET and NOTCH1 were reported to be associated with slicing defects [33]. Furthermore, different studies showed that single-nucleotide variations in splicing regulatory cis-elements lead to intron retentions, particularly in tumor suppressor genes, including ARID1A, PTEN, and TP53 [33] as well as to exon splicing alterations in proto-oncogenes, such as PDGFRA and EGFR [33,34].

Besides gene mutations, dysregulation of splicing factors through expression and/or activity alteration has commonly been observed and significantly contributes to aberrant splicing in cancer [33]. The mechanisms, nonetheless, are still poorly defined. As recently summarized by Wang and Colleagues [33], it was reported that several oncogenic signaling pathways (including EGFR, PI3K-AKT, MAPK, Wnt and signals from tumor microenvironment) might modulate the activity of the splicing machinery through different mechanisms, like transcriptional regulation, and/or post-translational modification [33]. It is noteworthy that PDGFRA signaling, found to be aberrantly active in many PTCL types might be on the one side responsible for spicing machinery misfunction and, on the other side, aberrantly expressed itself due to aberrant splicing. Further studies are needed, however, to better elucidate the interplay between onco-signals and spicing factors in lymphomas

and cancers more generally. In conclusion, our study showed for the first time that SF3B1 is not genetically altered in PTCL/NOS. Future studies are warranted to better define the bases of the molecular pathogenesis of this orphan disease.

Funding

This work was supported by AIRC (IG 2013 N.14355, Prof Piccaluga), Centro Interdipartimentale per la Ricerca sul Cancro "G. Prodi", BolognAIL, RFO (Prof. Piccaluga), FIRB Futura 2011 RBFR12D1CB (Prof. Piccaluga); Cariverona Foundation, ENACT project VIRO-COVID (Prof. Gibellini). The authors have no conflicting financial interests to declare.

Acknowledgements

The Authors are grateful to Dr. Maria Rosaria Sapienza and Dr. Maria Antonella Laginestra for the technical assistance.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2021.37.005944

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