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The relationship of REL proto-oncogene to pathobiology and chemoresistance in follicular and transformed follicular lymphoma

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ABSTRACT

Follicular lymphoma (FL) is a common type of indolent lymphoma that occasionally transforms to more aggressive B-cell lymphomas. These transformed follicular lymphomas (tFL) are often associated with chemoresistance whose mechanisms are currently unknown. *REL*, a proto-oncogene located on frequently amplified 2p16.1-p15 locus, promotes tumorigenesis in many cancer types through deregulation of the NF-κB pathway; however, its role in FL pathobiology or chemoresistance has not been addressed. Here, we evaluated *REL* gene copy number by q-PCR on FFPE FL tumor samples, and observed *REL* amplification in 30.4% of FL cases that was associated with weak elevation of transcript levels. PCR-Sanger analysis did not show any somatic mutation in FL tumors. In support of a marginal oncogenic role, a REL-transduced FL cell line was positively selected under limiting serum conditions. Interestingly, reanalysis of previously reported gene expression profiles revealed significant enrichment of DNA damage-induced repair and cell cycle arrest pathways in tFL tumors with high REL expression compared to those with low REL expression consistent with the critical role of c-REL in genotoxicity-induced NF-κB signaling, which was reported to lead to drug resistance. In addition to DNA damage repair genes such as ATM and BRCA1, anti-apoptotic BCL2 was significantly elevated in REL-high FL and tFL tumors. Altogether these data suggest that other genes located in amplified 2p16.1-p15 locus may have more oncogenic role in FL etiology; however, high REL expression may be useful as a predictive biomarker of response to immunochemotherapy, and inhibition of c-REL may potentially sensitize resistant FL or tFL cells to chemotherapy.

1. Introduction

Follicular lymphoma (FL) is the most common indolent lymphoma in the world [1]. It has a heterogeneous clinical course with ~10 year median survival after diagnosis [2]. Histological transformation (HT) to more aggressive malignancies collectively called as transformed follicular lymphomas (tFLs) such as diffuse large B-cell lymphoma (DLBCL) or Burkitt's lymphoma (BL) is observed in 10–60% of the FL patients [3]. Transformed follicular lymphomas (tFLs) are associated with shorter overall survival and immunochemotherapy resistance [4].

Neoplastic transformation of germinal center (GC) B cells are considered to be responsible for FL tumorigenesis [5]. t(14;18)(q32;q21) translocation contributes to neoplastic transformation of GC B cells in majority of the cases by bringing the IgH enhancer upstream of BCL2 [6] and thereby upregulating BCL2 expression [7], a pro-survival gene known to inhibit apoptosis [8]. Studies performed in BCL2 transgenic mice revealed that overexpression of BCL2 is not enough –by itself- suggesting other oncogenic events

Abbreviations: ECOG, Eastern Cooperative Oncology Group; FL, follicular lymphoma; FFPE, formalin-fixed paraffin-embedded; IPI, international prognostic index; LDH, lactate dehydrogenase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PMIG, pMSCV-IRES-GFP; tFL, transformed follicular lymphoma.

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are needed for complete establishment of FL [9]. A recent study that included whole-exome sequencing on 10 FL-tFL tumor pair revealed recurrent mutations of genes in chromatin modifiers, JAK-STAT or NF-κB pathway [10] suggesting that deregulation of these pathways may have a role in initiation or progression of FL tumors.

REL amplification is frequently observed in many lymphoma types including DLBCL [11], classical Hodgkin's lymphoma [12] or primary mediastinal B-cell lymphoma [13]. On the other hand, copy number variation analysis of FL or tFL tumors showed discrepant results with respect to the presence of amplification of 2p16.1-p15 locus that includes *REL* [14–16]. A recent report based on high-throughput SNP array analysis of CNVs in FL tumor samples revealed that 22.2% of FL and 35.6% of tFL cases, have 2p16.1-p15 gain/amplification that involves *BCL11A* in addition to *REL* proto-oncogene [17]. Altogether, these studies suggest that there may be one or more oncogene candidate in this recurrently amplified locus. However, whether REL contributes to FL pathogenesis has not been addressed previously.

c-REL (protein encoded by *REL* gene) is a transcription factor, and acts as a mediator of the NF-κB signaling pathway by forming dimers with other REL family members or with each other [18]. In B-cells, c-REL was shown to promote proliferation and survival [19]. Its overexpression/activation has been shown to contribute to neoplastic transformation in several solid tumors or hematologic malignancies [20]. In addition, c-REL overexpression was shown to correlate with chemotherapy resistance in serous epithelial ovarian cancer [21]. Indeed, previous reports showed that NF-κB pathway activation may be responsible for chemotherapy resistance in many cancer types including cervical carcinoma [22] and gastric cancer [23].

Here we showed frequent amplification of *REL* in FL tumors, and evaluated whether any association is present between amplification and clinical variables. We also showed moderate positive selection of REL-transduced FL cell line under limiting serum conditions. More importantly, we observed that tFL tumors with high REL expression has activation of DNA damage-induced cell cycle arrest and repair pathways, with upregulation of ATM, BRCA1 and BCL2, which may be a useful predictive biomarker of response to immunochemotherapy or therapeutic target for overcoming chemoresistance in these tumors.

2. Materials and methods

2.1. Patient samples and cell lines

Twenty-five follicular lymphoma tumor biopsies used in this study were collected at West China Sichuan University Hospital during routine diagnosis. The cases were reviewed by at least two hematopathologists with consensus diagnosis. Four μM thick sections were obtained from tissue blocks of FL biopsies fixed with buffered formalin and embedded in paraffin (FFPE). For each patient, the diagnostic tumor sample was mounted on each of two slides for DNA or RNA isolation, and stored at 4 °C to decrease the possibility of nucleic acid degradation. Histopathological and clinical characteristics of FL patients of this study are shown in Table S1. Additionally, publicly available data [17] of 42 tFL patients whose biopsies were collected by the Lymphoma/Leukemia Molecular Profiling Project (LLMPP) consortium or the University of Nebraska Medical Center (UNMC) were used in the current study. Diagnoses of these tFL cases were performed by a panel of LLMPP hematopathologists. A patient was diagnosed as tFL if a DLBCL occurred in the patient who was concurrently or previously diagnosed with FL. The transformed FL vs. high histopathological grade FL distinction was established by diagnosing a case as high grade (i.e. stage 3A or 3B) FL if a higher number of large cells were observed without loss of follicular histology. By contrast, a transformed FL case did no longer show follicular pattern and usually represented by a DLBCL. The characteristics of these cases are shown in Table S2.

KARPAS-422 [24], HEK293T and SU-DHL-4 [25] cell lines are gifts from Dr. Anna Scuto at City of Hope Medical Center. KARPAS-422 and SU-DHL-4 cell line were cultured in RPMI medium supplemented with 10% FBS, penicillin G (100 units/mL), streptomycin (100 μg/mL), and L-glutamine. Cell culture medium used for HEK393T cells was the same except that DMEM was used instead of RPMI. All cells were kept at a humidified incubator at 37 °C in 5% CO_2.

2.2. Evaluation of REL gene copy number with q-PCR

REL copy number analysis of FFPE FL tumors was performed with quantitative real-time PCR (qPCR) using primers designed against the *REL* genomic DNA isolated with QIAamp DNA FFPE Tissue Kit (Qiagen Inc., Germany) by applying the same qPCR-based method used earlier for the detection of mono-allelic deletion of *HACE1* [26] in natural killer/T cell lymphomas. Forty ng genomic DNA was used as template for q-PCR reactions that were performed with DyNAmo HS SYBR Green qPCR Kit master mix (ThermoFisher Scientific Inc.) using Roche LightCycler480 thermal cycler. ΔΔCt method was used for quantification of copy numbers. Gene copy number of *REL* was calibrated to that of *RPS13* reference gene [27]. Then, *REL* genomic copy number of FL tumors was normalized to that of normal human NK cells activated with IL2 for 3 days, which was available from a previous study [26]. The cut-off to define *REL* gain/amplification was set as two-fold increase. The *REL* and *RPS13* genomic q-PCR primers used for copy number analysis are as follows: REL genomic q- PCR forward: 5′-CCCTTGGTAACAGAATCCCTATT- 3′, REL genomic q-PCR reverse: 5′-CTGACTTTCAACTGGGCCTTA-3′; RPS13 genomic q-PCR forward: 5′-CGACGTGAAGGAGCAGATTTA-3′; RPS13 genomic q-PCR reverse: 5′-CACGAGGACAGGCGAAATAG-3′.

2.3. Comparison of copy number and mRNA expression of REL in transformed follicular lymphomas

Copy number and gene expression values of REL were obtained for 42 tFL cases with both SNP array (Affymetrix Mapping 250 K Nsp SNP Array) and DNA microarray (GeneChip Human Genome U133 Plus version 2.0) data available in Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) database with the following accession numbers: GSE81183 and GSE81184. Log2 intensities of copy number variation data of SNP_A-2301401 and SNP_A-2094019 probe intensities determined with Affymetrix Genotyping Console software were used to estimate copy number of *REL*. SNP_A-2301401 and SNP_A-2094019 correspond to rs12474254 and rs13419008 single nucleotide polymorphisms (SNPs), respectively, and they are located ~43 kb and ~81 kb upstream of the human *REL* gene based on the UCSC Genome Browser (hg38). Intensity values of these SNP probes were highly concordant (Fig. S1) and their means were used to estimate *REL* copy number in 42 tFL tumors.

REL mRNA expression of 42 tFL cases in the NCBI GEO database (accession code: GSE81184) was analyzed with NCBI GEO2R gene expression analysis tool [28] using log2-normalized median centered gene expression profile (GEP) data.

2.4. Generation and ectopic expression of c-REL in a FL cell line and evaluation of cell growth

The directional cloning procedure followed to clone REL coding sequence into MSCV-IRES-GFP (PMIG) is as follows: REL coding sequence was amplified from pcDNA-REL-FLAG (Addgene plasmid