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AUTHOR'S VIEWS

Interaction between somatic mutations and germline variants contributes to clinical heterogeneity in cancer

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ABSTRACT

Deciphering principles of inter-tumoral heterogeneity is crucial for refinement of precision oncology. We have recently demonstrated that 'oncogenic cooperation' between somatic mutations and regulatory germline variants can serve as a major cause for inter-tumoral heterogeneity, suggesting the requirement of integrating the regulatory genome into 'omics'-based precision oncology.

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The increasing availability and technological advancements of 'omics'-technologies in the past years progressively enabled the individualization of diagnosis and treatment of diseases – an approach often referred to as 'precision' or 'personalized' medicine.[1] In this regard, oncology holds an avantgarde position in relation to other medical disciplines: based on somatic mutations in the protein-coding genome, patients can be subjected to targeted therapies.[1]

However, compared to cancers of adulthood, pediatric cancers are characterized by a strikingly low number of recurrent somatic mutations.[2] These few somatic alterations may not explain the high inter-individual variability in tumor behavior and clinical outcomes observed,[2,3] and render current approaches of personalized oncology in many instances to be less effective in pediatric malignancies. Hence, it is important to uncover mechanisms underlying inter-individual variability in oligo-mutated cancers, to improve diagnosis, risk-prediction and targeted therapies in the context of precision oncology.[3]

In our recent study, we show in the Ewing sarcoma (EwS) model how the interplay between somatic mutations and regulatory germline variants impacts on tumor growth, patient survival, and drug response, thereby constituting a major cause of inter-individual tumor heterogeneity[4] (Figure 1).

In EwS, the pathognomonic fusion transcription factor Ewing sarcoma breakpoint region 1-Friend leukemia integration 1 (EWSR1-FLI1) can bind to highly polymorphic GGAA-microsatellites (mSats). EWSR1-FLI1-binding to such mSats can convert them into active enhancers, whereby the number of consecutive GGAA-repeats modulates the enhancer activity of the respective mSat.[5–7] Indeed, it has been shown that the interplay between EWSR1-FLI1 and such GGAA-mSats may influence the inter-individual susceptibility toward EwS tumorigenesis.[6,7] However, whether such cooperation may also influence the inter-individual variability in tumor progression and therapy response remained unclear.

To identify clinically relevant EWSR1-FLI1 target genes that may potentially mediate inter-tumoral heterogeneity, we screened in a first step available 'omics'-datasets for genes that are on the one hand regulated by EWSR1-FLI1, and on the other hand associated with worse overall patient survival when being highly expressed.[4] This analysis revealed *MYB proto-oncogene like 2* (*MYBL2*), encoding for a transcription factor involved in regulation of cell cycle, cell survival and cell differentiation,[8] as the top hit of our screen.[4] In a second step we analyzed published EWSR1-FLI1 chromatin immunoprecipitation and sequencing (ChIP-seq) data for peaks nearby MYBL2, which displayed histone marks indicative for active enhancers. One of the most prominent peaks mapped to a polymorphic GGAA-mSat located about 150kb telomeric of the *MYBL2* gene.[4] Using reporter assays, we validated the EWSR1-FLI1-dependent enhancer activity of this GGAA-mSat *in vitro* and confirmed its regulatory effect on *MYBL2* expression by Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi).[4] The regulatory potential of this GGAA-mSat on *MYBL2* transcription was further supported by its expression quantitative trait loci (eQTL) properties.[4] In fact, we found in a whole-genome sequencing dataset comprising 35 primary EwS tumors with matched gene expression data, that EwS tumors exhibiting ≤13 GGAA-repeats at both alleles of the *MYBL2*-associated GGAA-mSat exhibited significantly lower *MYBL2* mRNA levels than those with >13 GGAA-repeats at both alleles.[4] Interestingly, analysis of 38 whole-genome sequenced matched tumor/germline pairs revealed that the number of

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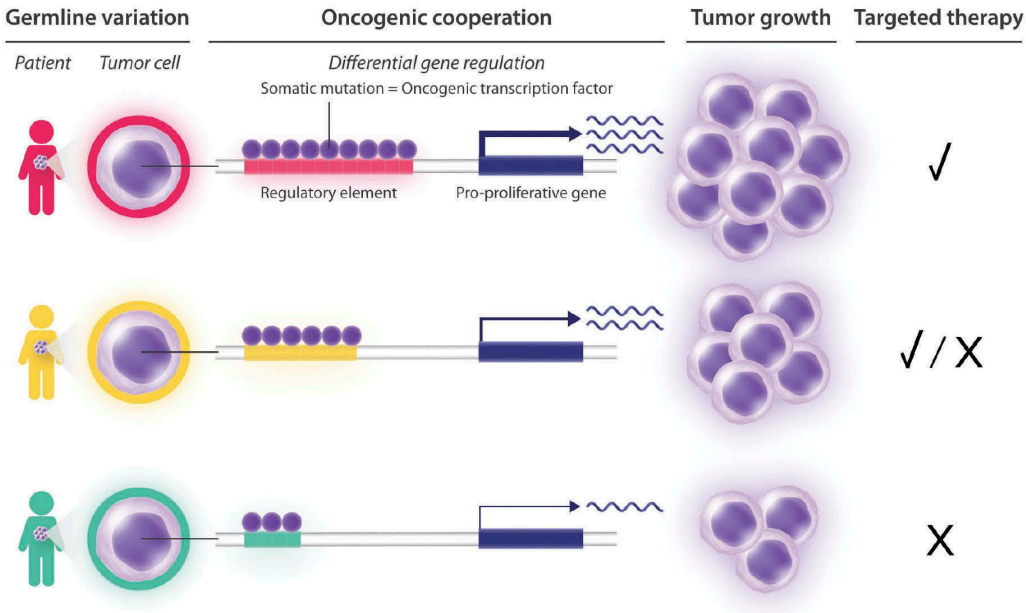

Figure 1. Oncogenic cooperation determines inter-individual heterogeneity in tumor growth and drug response. Variability of a regulatory element inherited via the germline modifies the effect of a disease-driving somatic mutation and influences targeted therapy effectivity.

consecutive GGAA-repeats at the *MYBL2*-associated mSat was entirely conserved at both alleles in every matched tumor/germline pair, indicating that the mSat haplotypes are inherited via the germline.[4] Using RNA interference (RNAi) experiments, we characterized the functional role of MYBL2 *in vitro* and *in vivo*, demonstrating that MYBL2 suppression impairs cell proliferation (accompanied by G2/M blockage), cell survival and clonogenic growth of EwS.[4] Through combination of RNA-sequencing data with and without MYBL2 knockdown, MYBL2 ChIP-seq data, gene expression data of primary EwS with matched clinical annotations, and functional experiments, we identified *cyclin F* (*CCNF), baculoviral IAP repeat-containing 5* (*BIRC5*) and *aurora kinase B* (*AURKB*) as critical, functionally and clinically relevant, MYBL2 target genes.[4] To therapeutically exploit our findings, we targeted cyclin dependent kinase 2 (CDK2), the upstream kinase phosphorylating and activating MYBL2.[4,8] Using small-molecule inhibitors, we showed that high MYBL2 levels sensitize EwS cells for anti-CDK2 treatment *in vitro* and *in vivo*, suggesting a potential use for MYBL2 as a predictive biomarker for effective CDK2 inhibitor treatment.[4]

In summary, our findings made in the EwS model exemplify how oncogenic cooperation of a somatic driver-mutation (here EWSR1-FLI1) and a regulatory germline variant (here a polymorphic enhancer-like GGAA-mSat) may essentially determine inter-tumoral heterogeneity by influencing tumor growth, drug response and patient survival through modulation of a critical druggable downstream player (here MYBL2).[4] These findings possibly represent a general principle that accounts for inter-individual variations in disease phenotypes, which is supported by recent data from research fields other than oncology showing that the same somatic event or mutation may result in different phenotypes depending on (inherited) regulatory element variations.[9–11] For example, it has been shown in *Caenorhabditis elegans* (*C. elegans*) that the severity of RNAi phenotypes differs depending on variations between the genetic backgrounds of two particular *C. elegans* isolates.[9] Similarly, in mice, single nucleotide polymorphisms (SNPs) in binding sites of the peroxisome proliferator-activated receptor gamma (PPARγ) transcription factor modify the function of PPARγ as well as response to anti-diabetic drugs,[11] and in humans, a genome-wide association study (GWAS) identified genetic variants modifying the onset of Huntington's disease.[10] In this respect, after identification of disease-specific unfavorable regulatory variants interacting with dominant driver-oncogenes, future approaches in precision oncology specifically could aim at sequencing also non-coding regulatory genomic regions to stratify patients into risk-groups according to their genetic context (i.e. the pattern of genetic regulatory variants with which somatic mutations cooperate). Thus, we anticipate that our findings are translatable to other cancer entities beyond EwS, and suggest to include the regulatory genome in approaches of 'omics'-based precision oncology.[4]

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Disclosure of potential conflicts of interest

The authors declare no conflict of interests.

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