MULTI-OMIC AND MULTI-REGION PROFILING OF DESMOID TUMORS REVEALS INTRA- AND INTER-TUMOR HETEROGENEITY

Chelsea De Bellis[1], Sujay Vennam[2], Christopher Eeles[3], Justin Cates[4], Thomas Stricker[5], Kristen Ganjoo[6], Benjamin Haibe-Kains[3,7,8], Matt van de Rijn[2], **Joanna Przybyl** [1,9]

1 Department of Surgery, McGill University, Montreal, QC, Canada
2 Department of Pathology, Stanford University School of Medicine, Stanford, CA, USA
3 Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada
4 PathGroup Laboratories, Nashville, TN, USA
5 One Oncology, Nashville TN, USA
6 Department of Medicine, Division of Medical Oncology, Stanford University, Stanford, CA, USA
7 Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada
8 Vector Institute for Artificial Intelligence, Toronto, ON, Canada
9 Cancer Research Program, The Research Institute of the McGill University Health Centre, Montreal, QC, Canada

INTRODUCTION

Desmoid tumors are histologically bland fibroblastic tumors that do not metastasize but have a high rate of local recurrence. Currently, no reliable predictors of clinical behavior exist. Previously published studies proposed two different transcriptomic signatures to predict relapse (Salas et al. 2015, Kohsaka et al. 2021). However, these two transcriptomic signatures had no overlapping genes or represented pathways, and neither of them have been introduced into routine clinical practice. In this study, we sought to develop a new prognostic signature for desmoid tumors and validate the previously published signatures. This led us to a comprehensive multi-omic investigation of the possible molecular intra- and inter-tumor heterogeneity of desmoid tumors.

METHODS

We performed transcriptomic profiling of 31 specimens from 20 primary desmoid tumors, and in-depth multi-omic analysis including DNA methylation, DNA copy number alterations, point mutations and gene expression on 24 specimens from different regions of primary and recurrent desmoid tumors obtained from 3 patients (7 - 9 specimens per patient).

RESULTS

We observed highly variable expression of transcriptomic prognostic signatures, both in patients who did or did not progress (**Fig. 1**). Signatures associated with favorable and unfavorable outcome were detected in different regions within the same tumor.

Further multi-omic studies in selected patients showed remarkable intra- and inter-tumor heterogeneity of genomic, epigenomic and transcriptomic patterns. Through reconstruction of phylogenetic trees based on single nucleotide variants, we identified novel clonal and subclonal somatic mutations (**Fig. 2**). In one patient, we detected two co-existing *CTNNB1* mutations in different regions of the same recurrent tumor (**Fig. 2**). Overall, the transcriptomic profiles showed the highest degree of variability within tumors and between primary and recurrent tumors from the same patient.

CONCLUSIONS

Our study demonstrates a substantial inter-patient and intra-tumor heterogeneity of expression of transcriptomic prognostic signatures. Our study shows an unexpected degree of intra- and inter-tumor heterogeneity in desmoid tumors. Our analysis indicates that molecular analysis of a single tumor biopsy may underestimate the magnitude of molecular alterations in desmoid tumors. Our study also shows that recurrent desmoid tumors acquire multiple new molecular alterations. Molecular heterogeneity is an important consideration in drug development and validation of prognostic and predictive biomarkers for desmoid tumors.

FIGURES

FIGURE 1. Heterogeneity of expression of prognostic signatures in desmoid tumors. Heatmaps demonstrating expression levels of genes included in the prognostic transcriptomic signatures developed in (A) our study and in two previously published studies by (B) Salas et al. (PMID: 25878329) and (C) Kohsaka et al. (PMID: 33444924).

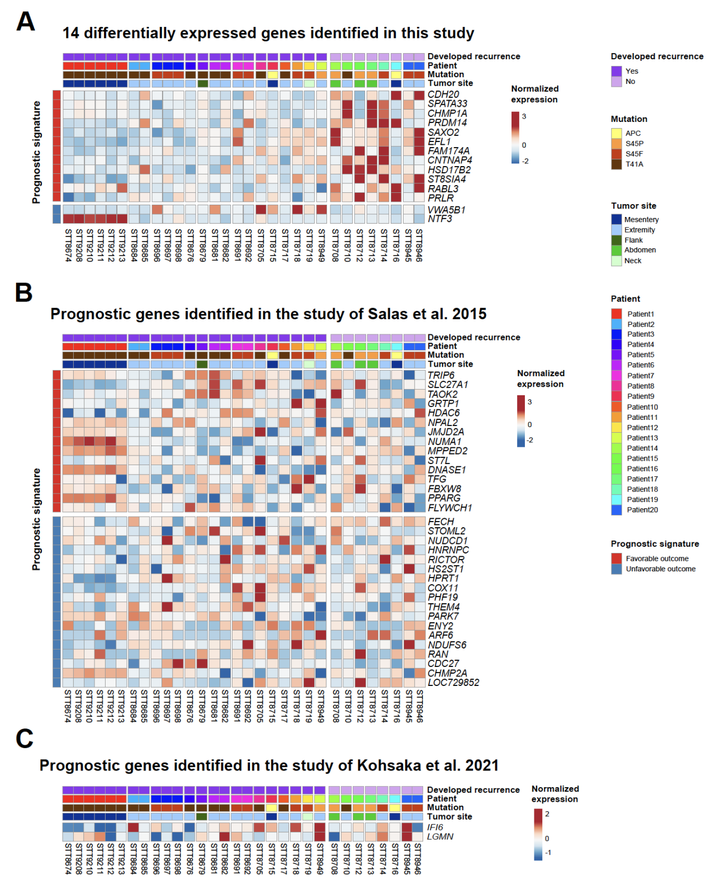
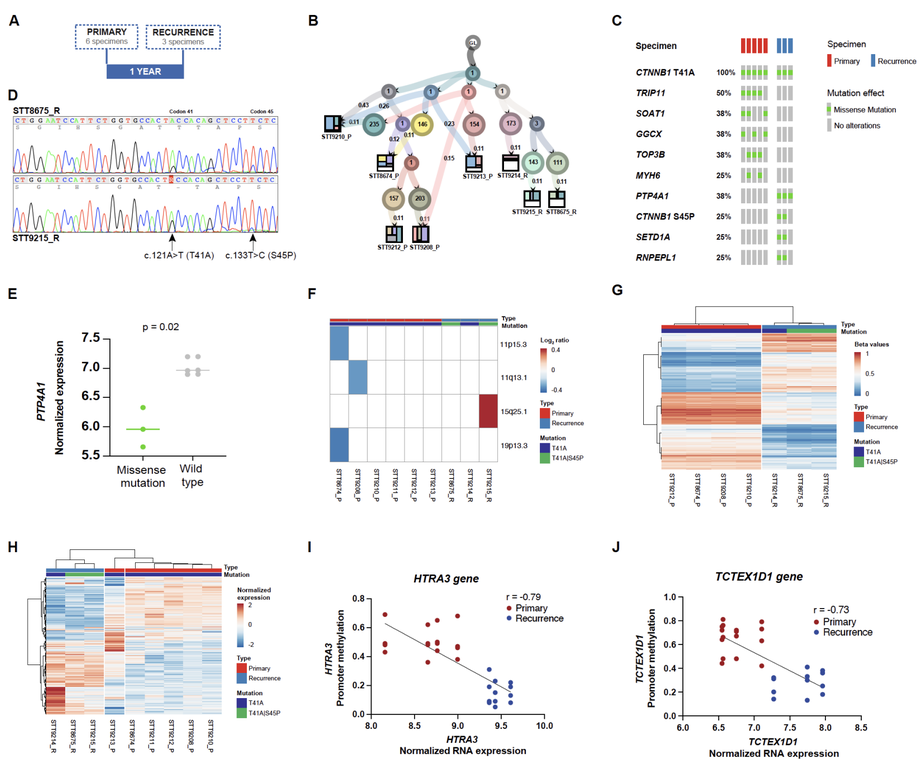


FIGURE 2. Genomic phylogeny and multi-omic tumor heterogeneity in Patient 1. **(A)** Specimens analyzed from Patient 1. **(B)** Reconstruction of the phylogenetic tree based on non-synonymous somatic mutations. **(C)** Oncoprint representing clonal and subclonal mutations seen in the genomic phylogenetic tree. **(D)** Validation of the presence of two distinct mutations in the *CTNNB1* gene in two regions of the recurrent tumor. Sanger sequencing results indicating the co-existing T41A and S45P mutations. **(E)** Normalized gene expression levels of the *PTP4A1* gene in tumor specimens carrying missense mutation and wild type sequence of this gene in Patient 1. **(F)** DNA copy number alterations detected in the primary and recurrent tumor. **(G,H)** Unsupervised clustering of different regions of the primary and recurrent tumors based on the top 1% most variable CpGs **(G)** and normalized RNA expression of the top 10% most variable genes **(H)**. **(I,J)** Correlation between beta values representing DNA methylation in the promoter region and normalized gene expression of the **(I)** *HTRA3* and **(J)** *TCTEX1D1* genes.



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