Circular RNA profiling distinguishes medulloblastoma groups and shows aberrant RMST overexpression in WNT medulloblastoma

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CORRESPONDENCE

Circular RNA profiling distinguishes medulloblastoma groups and shows aberrant *RMST* overexpression in WNT medulloblastoma

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Medulloblastoma is the most common malignant brain tumor in childhood [7]. Molecular classification into WNT, SHH, Group 3, and Group 4 of medulloblastoma refines surveillance of tumor predisposition syndromes, improves risk stratification to (de-)escalate therapeutic interventions, and sets the stage for targeted therapies [7]. Discrimination between Group 3 and Group 4 medulloblastoma remains challenging with partly inconsistent group annotation using DNA methylation data [1], gene expression profiling [8] or (phospho-)proteomics [3]. Thus, studies are increasingly integrating two or more molecular layers for accurate group assessment of this heterogeneous disease [1, 8].

Circular RNAs (circRNA) recently emerged as promising biomarkers with cell type- and developmental stage-specific expression patterns in human tissues and cancers [10]. They are not easily degraded by exonuclease RNase R, are long-lived and are widely detected in body fluids [10]. Based on these unique properties, circRNAs may serve as clinically useful diagnostic biomarkers. We developed a bioinformatic approach called "circs", combining three circRNA detection pipelines (find_circ [5], DCC [2], and CIRCexplorer [11]) reducing the false-positive rate compared to single in silico circRNA detection method.

We could detect circRNAs with higher expression in medulloblastoma (Figures S1a and S1b, Table S1), but most circRNAs were significantly lower expressed compared to fetal brain tissue samples as previously reported (Fig. 1a) [10]. In contrast to the previous indications that non-coding RNA expression profiles were not sufficient to distinguish groups [9], unsupervised hierarchical clustering revealed the four core groups using the top 500 most differentially expressed circRNAs in our discovery cohort ($n=38$, Fig. 1b, Table S2), and in our non-overlapping validation cohort ($n=35$; Fig. 1c, Table S3). We confirmed medulloblastoma classification of the discovery cohort based on the previous similarity network fusion (SNF) analysis (Fig. 1d, data

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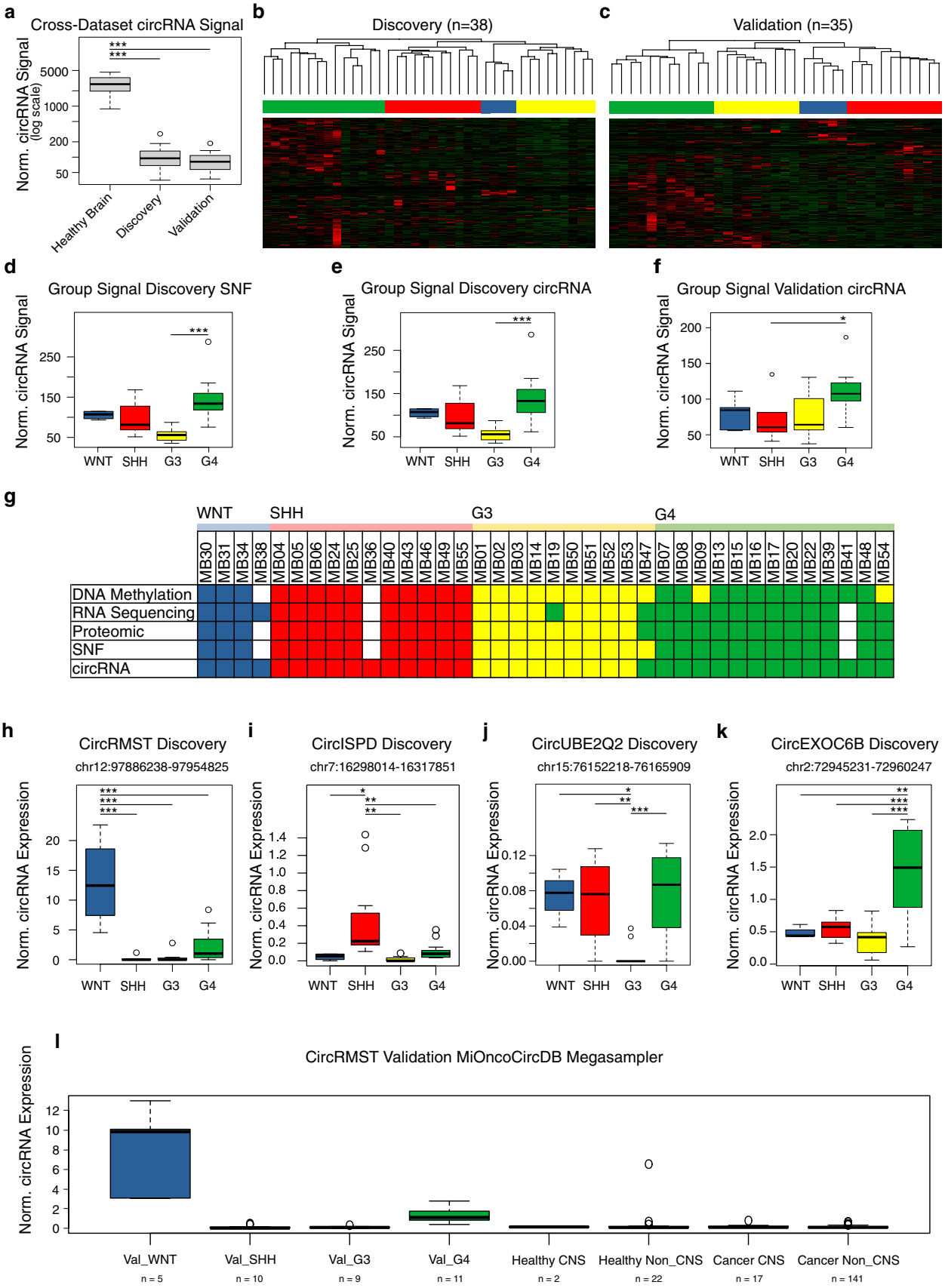
a
Cross-Dataset circRNA Signal
Norm. circRNA Signal (log scale)
5000
1000
200
50
***
***
Healthy Brain
Discovery
Validation
b
Discovery (n=38)
c
Validation (n=35)
d
Group Signal Discovery SNF
Norm. circRNA Signal
250
150
50
***
WNT SHH G3 G4
e
Group Signal Discovery circRNA
Norm. circRNA Signal
250
150
50
***
WNT SHH G3 G4
f
Group Signal Validation circRNA
Norm. circRNA Signal
200
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WNT SHH G3 G4
g
WNT
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G3
G4
MB30 MB31 MB34 MB38 MB04 MB05 MB06 MB24 MB25 MB36 MB40 MB43 MB46 MB49 MB55 MB01 MB02 MB03 MB14 MB19 MB50 MB51 MB52 MB53 MB47 MB07 MB08 MB09 MB13 MB15 MB16 MB17 MB20 MB22 MB39 MB41 MB48 MB54
DNA Methylation
RNA Sequencing
Proteomic
SNF
circRNA
h
CircRMST Discovery
chr12:97886238-97954825
Norm. circRNA Expression
20
15
10
5
0
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WNT SHH G3 G4
i
CircISPD Discovery
chr7:16298014-16317851
Norm. circRNA Expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
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WNT SHH G3 G4
j
CircUBE2Q2 Discovery
chr15:76152218-76165909
Norm. circRNA Expression
0.12
0.08
0.04
0.00
*
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WNT SHH G3 G4
k
CircEXOC6B Discovery
chr2:72945231-72960247
Norm. circRNA Expression
2.0
1.5
1.0
0.5
0.0
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WNT SHH G3 G4
l
CircRMST Validation MiOncoCircDB Megasampler
Norm. circRNA Expression
12
10
8
6
4
2
0
Val_WNT n = 5
Val_SHH n = 10
Val_G3 n = 9
Val_G4 n = 11
Healthy CNS n = 2
Healthy Non_CNS n = 22
Cancer CNS n = 17
Cancer Non_CNS n = 141

◂**Fig. 1** Circular RNA expression profiles define medulloblastoma groups. **a** Circular RNA (circRNA) signal across fetal brain controls (healthy, $n=12$), discovery ($n=38$) and validation medulloblastoma (MB) cohort ($n=35$). **b**, **c** Heatmap of top 500 differentially expressed circRNAs in discovery (**b**) and in validation data set (**c**), hierarchical clustering by average Pearson dissimilarity, circRNA MB groups: *WNT* blue, *SHH* red, *G3* yellow, *G4* green. **d** Circular RNA signals across discovery data set similarity network fusion (SNF) MB groups. **e** Circular RNA signal across discovery data set (circRNA MB groups). **f** Circular RNA signal across validation data set (circRNA MB groups). **g** Medulloblastoma grouping according to [3] and circRNA data in discovery data set. MB36 was diagnosed as SHH-MB in the routine diagnostic setting. White square = data not available. **h–k**. Boxplot of circRMST, circISPD, circUBE2Q2, and circEXOC6B in discovery data set with circRNA-based groups and genomic coordinates. **l** Megasampler with normalized circRMST expression in validation data set with the circRNA groups (Val_WNT = WNT validation data set; Val_SHH = SHH validation data set; Val_G3 = G3 validation data set; Val_G4 = G4 validation data set) and MiOncoCircDB (four categories: healthy_CNS, healthy_non_CNS, cancer_CNS, cancer_non_CNS, details see Tables S9 and S10). Tukey's HSD adjusted p values: $*p<0.05$; $**p<0.01$; $***p<0.001$

obtained from methylome, transcriptome, and proteome [3]) and using circRNA-based grouping in our discovery cohort (Fig. 1e) and in our validation cohort (Fig. 1f).

Using only circRNA expression profiles of the top 500 most differentially expressed circRNAs in our discovery cohort, we were able to reliably separate the four core groups almost as accurately as using SNF integrating multi-omics data (34/35 = 97.14% concordance; adjusted rand index = 0.91, $p=0.001$; Fig. 1g).

To discern group-specific and subtype-specific circRNA biomarkers, we performed differential expression analysis of the circRNA-defined groups (Fig. 1h–k, Figures S2a–e, S3a–d) and of DNA methylation-defined subtypes (Figure S4a–d, Table S4). Significant and consistent up- or down-regulations of circRNAs were identified in both cohorts for WNT ($n=81$; Table S5, Figs. 1h, S2a, S3a), SHH ($n=7$; Table S6, Figs. 1i, S2b, S3b), and Group 4 ($n=13$; Table S7, Figs. 1k, S2d, S3d), while Group 3-specific circRNAs were not consistently detected (Table S8, Figs. 1j, S2c, S3c). Notably, a circRNA derived from the *rhabdomyosarcoma 2-associated transcript* (*RMST*) locus (chr12: 97886238–97954825) was aberrantly overexpressed in WNT medulloblastomas compared to the other groups ($p<0.001$; Figs. 1h, S2a). *RMST* is a well-known long non-coding RNA, which is exclusively expressed in brain tissue [6], and mostly present in circular isoforms [4]. Using a published cohort MiOncoCircDB consisting of circRNAs across over 2000 cancer samples [10], we validated circRMST as a highly reproducible and specific biomarker for WNT medulloblastoma (Fig. 1I).

In conclusion, we demonstrate a powerful and reliable method for molecular classification using our pipeline *circs* without the necessity to include additional information layers. Thus, circRNA-based biomarkers may help to improve diagnostic and therapeutic approaches in this highly aggressive disease.

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