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The RNA-binding protein IGF2BP1 regulates stability of mRNA transcribed from FOXM1 target genes in hypermitotic meningiomas

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Meningiomas, the most common primary intracranial tumors, usually follow an indolent clinical course [1]. However, a subset of aggressive meningiomas frequently recur despite multimodal treatments. DNA methylation signatures have recently been adopted as prognostic markers for meningioma recurrence [3, 9, 10], including an unsupervised approach that classifies meningiomas into three clinically distinct groups: merlin-intact (low-risk), immune-enriched (intermediate-risk), and hypermitotic (high-risk) [3]. Differences in RNA expression across meningioma molecular groups are further linked with risk of recurrence and response to post-operative radiotherapy [2]. These findings suggest that dysregulated RNA processing between aggressive and benign meningiomas may contribute to the biology of these tumors [6]. All aspects of RNA biology are regulated by RNA-binding proteins (RBPs), however, their role in shaping the transcriptome of meningiomas is largely unknown.

To determine if RBPs contribute to the biology of meningiomas, we quantified expression of 754 RBPs using RNA-seq data from 486 human meningiomas with matched DNA methylation profiles (Fig. 1a, online resource).

Comparing hypermitotic to merlin-intact meningiomas revealed 45 upregulated RBPs ($\text{Log}_2\text{FC} > 0.5$, $p_{\text{adj}} < 0.05$) and 45 downregulated RBPs ($\text{Log}_2\text{FC} < -0.5$, $p_{\text{adj}} < 0.05$). Compared to immune-enriched meningiomas, hypermitotic meningiomas had 18 upregulated and 41 downregulated RBPs. *IGF2BP1* was one of the most upregulated RBPs in hypermitotic meningiomas (hypermitotic vs merlin-intact $\text{Log}_2\text{FC} = 3.8$, $p_{\text{adj}} < 0.0001$; hypermitotic vs immune-enriched $\text{Log}_2\text{FC} = 1.6$, $p_{\text{adj}} < 0.001$) (Fig. 1b, c; Supplementary Table 1a, b). Individual meningioma's can have a large degree of intratumoral heterogeneity in gene expression [7]. We, therefore, examined heterogeneity in *IGF2BP1* expression using a spatial transcriptomic dataset with samples from all DNA methylation groups [7], identifying differences in *IGF2BP1* expression both within and between individual tumor samples (Supplementary Fig. 1a). IGF2BP1 acts predominantly as an mRNA stability factor and N6-methyladenosine (m6A) RNA binding protein with putative roles in tumorigenesis [5]. IGF2BP1 is expressed at relatively low levels in normal or tumor-adjacent tissues but overexpressed in corresponding tumor samples and is associated with poor prognosis across multiple tumor types

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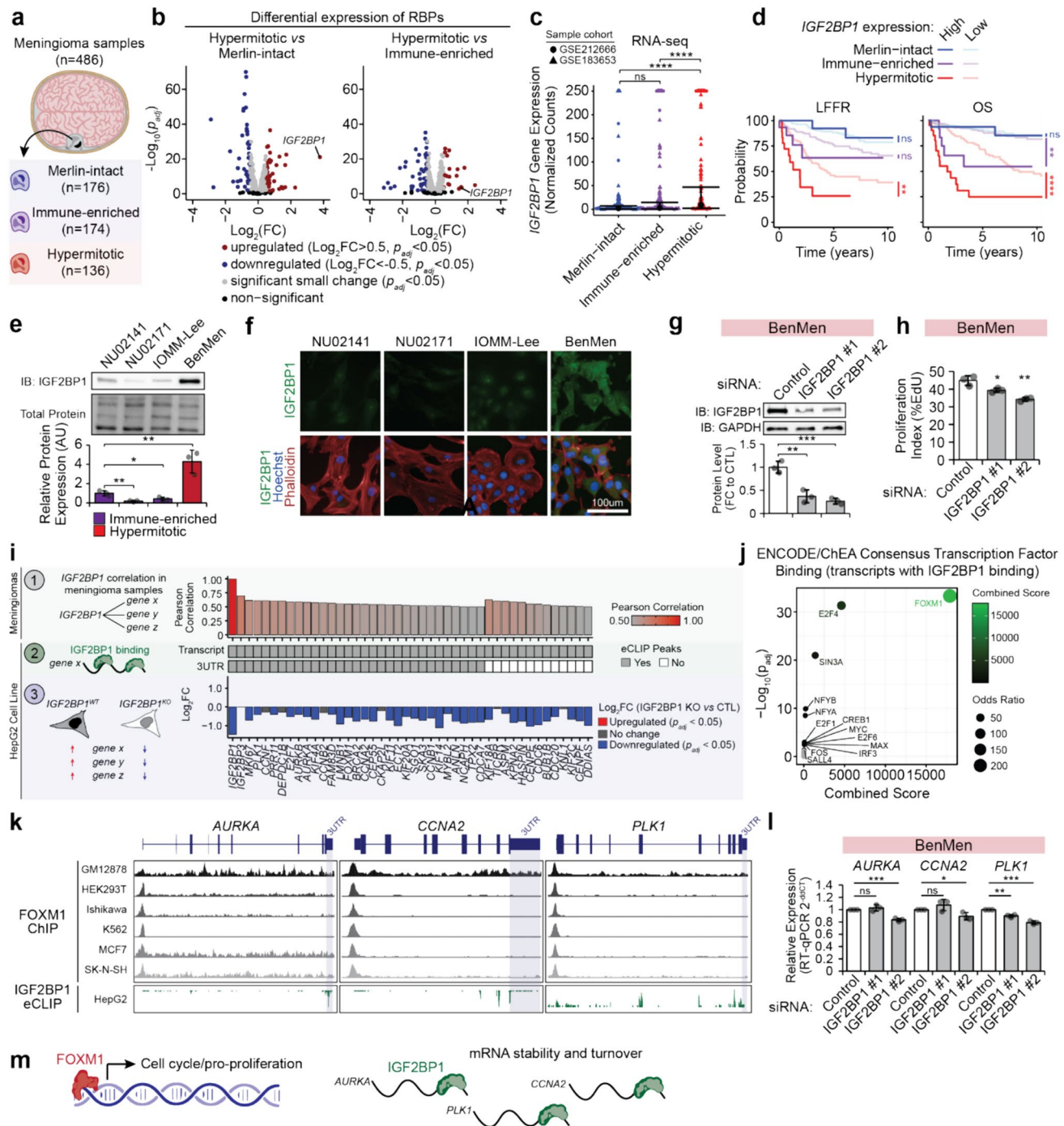
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[11]. In meningiomas, *IGF2BP1* methylation has been associated with tumor recurrence and more aggressive phenotypes [4, 14], but prior studies have not examined *IGF2BP1* expression or activity across meningioma DNA methylation groups. We stratified patients, first using DNA-methylation, and then by *IGF2BP1* expression and observed that patients with high *IGF2BP1* expression had significantly lower rates of overall survival (OS) and local freedom from recurrence (LFFR) (Fig. 1d). Additionally, upon regression analysis,

high *IGF2BP1* expression negatively impacted tumor control independent of other molecular and clinical variables (Supplementary Fig. 1b), together suggesting an association between *IGF2BP1* and outcomes.

To investigate the effect of *IGF2BP1* expression on meningioma cell biology, we used a hypermitotic meningioma cell line, BenMen, with ~4-fold higher *IGF2BP1* protein expression compared to three immune-enriched cell lines NU02141, NU02171, and IOMM-Lee (Fig. 1e, f) [3].

Fig. 1 IGF2BP1 is upregulated in hypermitotic meningiomas and promotes stability of FOXM1 target genes. **a** Meningiomas are grouped based on DNA-methylation profiles. **b** Differential expression of 754 RBPs between hypermitotic and merlin-intact meningiomas, or hypermitotic and immune-enriched meningiomas. Upregulated genes are in red ($\text{Log}_2\text{FC} > 0.5$, $p_{\text{adj}} < 0.05$), downregulated in blue ($\text{Log}_2\text{FC} < -0.5$, $p_{\text{adj}} < 0.05$), smaller significant changes in gray ($\text{Log}_2\text{FC} < |0.5|$, $p_{\text{adj}} < 0.05$). **c** *IGF2BP1* gene expression measured by RNA-seq across meningioma samples plotted as normalized counts by DNA methylation group ($n = 486$; median \pm IQR; Wilcoxon test, $**p < 0.01$, $***p < 0.0001$, ns—not significant). **d** Local freedom from recurrence (LFFR) and overall survival (OS) based on meningioma DNA-methylation and *IGF2BP1* expression level (z -score: high > 0 , low < 0). *IGF2BP1* protein expression in meningioma cell lines measured by western blot (**e**) and immunofluorescence (**f**). *IGF2BP1* levels are normalized to total protein, plotted as fold change relative to NU02141 ($n = 3$; mean \pm SD; t -test, $*p < 0.05$, $**p < 0.01$). Cells are counterstained with Hoechst and phalloidin (Scale bar 100 μm). *IGF2BP1* protein expression (**g**) and cell proliferation (**h**) in BenMen cells transfected with *IGF2BP1*-targeting siRNAs or a negative control 48 h post-transfection. *IGF2BP1* expression is measured by western blot and normalized to GAPDH loading control. Cell proliferation is measured using EdU incorporation, counterstained with Hoechst, plotted as percent EdU + to total Hoechst + cells ($n = 3$; mean \pm SD; t -test to control, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$). **i** 40 transcripts that correlate ($r > 0.5$) with *IGF2BP1* expression in meningiomas (top) are scored for the presence of *IGF2BP1* binding peak(s) using ENCODE HepG2 eCLIP data (middle), and their levels are measured in *IGF2BP1* Cas9-KO vs. control cells using ENCODE RNA-seq HepG2 data ($p_{\text{adj}} < 0.05$) (bottom). **j** Gene ontology analysis for transcription factor targets from ENCODE/ChEA using *IGF2BP1* target genes from (**i**) that correlate with *IGF2BP1* expression in meningiomas and contain *IGF2BP1* binding sites. **k** Binding peaks from FOXM1 ChIP-seq and *IGF2BP1* eCLIP-seq ENCODE assays, displayed as unique reads compared to control, in indicated cell lines for *AURKA*, *CCNA2*, and *PLK1* genes. **l** Expression of *AURKA*, *CCNA2*, *PLK1* in BenMen cells transfected with *IGF2BP1*-targeting siRNAs or a negative control at 48 h post-transfection assessed by RT-qPCR, normalized to *GAPDH* ($n = 3$; mean \pm SD; t -test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, ns—not significant). **m** Suggested model by which FOXM1 drives expression of cell cycle or pro-proliferative genes, which are then stabilized by *IGF2BP1* binding

siRNA-mediated *IGF2BP1* knock-down in BenMen cells resulted in a decrease in cell proliferation compared to control siRNA (Fig. 1g, h).

Since *IGF2BP1* primarily regulates mRNA stability through interactions with target RNA 3' untranslated regions (3'UTR) and m6A modifications [5], we examined how *IGF2BP1* impacts stability of target mRNAs in aggressive meningiomas. First, we identified transcripts whose expression was moderately correlated ($r > 0.5$) with *IGF2BP1* expression across meningiomas. We, then, filtered this list for any transcript with evidence of direct *IGF2BP1* binding, determined using publicly available ENCODE data from enhanced crosslinking and immunoprecipitation (eCLIP) assays in HepG2 hepatocellular carcinoma cells [12]. We identified 44 target RNAs from meningiomas that contained at least one eCLIP peak for *IGF2BP1*, including 31 (70%) containing at least one peak within the 3'UTR (Fig. 1i; Supplementary Table 1c). Of

the remaining transcripts without an *IGF2BP1* eCLIP peak, 38% were not expressed in HepG2 cells. Furthermore, 37 (84%) of target RNAs from meningiomas, including 26 out of 31 targets with 3'UTR peaks, were significantly downregulated following *IGF2BP1* knockout in HepG2 cells (Fig. 1i; Supplementary Table 1c). Together, these findings suggest that *IGF2BP1* binding to these target RNAs may promote transcript stability.

To further investigate the biological significance of *IGF2BP1* RNA targets, we performed gene ontology analysis and observed a significant enrichment in targets of FOXM1 (Fig. 1j; Supplementary Table 1d), a transcription factor that drives meningioma aggressiveness [13] and is enriched in hypermitotic meningiomas [3]. These targets included genes associated with cell-cycle control, e.g., *AURKA*, *CCNA2*, and *PLK1*, and their expression positively correlated with *IGF2BP1* expression across meningioma samples (Supplementary Fig. 1c). Within individual tumor samples from the spatial transcriptomics dataset, regions of high *IGF2BP1* expression also exhibited high *AURKA*, *CCNA2*, and *PLK1* expression (Supplementary Fig. 1d). Expression of these genes are enriched in BenMen Hypermitotic cells, as well as in IOMM-Lee, an Immune-enriched cell line with a high proliferative rate in vitro, compared to other Immune-enriched cell lines (NU02141 and NU02171) (Supplementary Fig. 1e). Analysis of available ENCODE data revealed all three genes contained both FOXM1 ChIP-seq peaks in their promoter regions and *IGF2BP1* eCLIP peaks within their coding and 3'UTR regions (Fig. 1k). Finally, siRNA-mediated knock-down of *IGF2BP1* resulted in significantly decreased expression of *AURKA*, *CCNA2*, and *PLK1* in BenMen cells (Fig. 1l), further suggesting their expression is regulated by *IGF2BP1*.

Together, these data support a role for the RBP *IGF2BP1* in regulating meningioma cell growth and suggests a model whereby *IGF2BP1* regulates the expression of cell cycle related genes downstream of FOXM1 in aggressive meningiomas (Fig. 1m), potentially through direct binding of *IGF2BP1* to its target transcripts. However, further functional validations are needed to confirm the model described here. Interestingly, individual meningiomas can have a wide degree of intratumoral variability in *IGF2BP1* expression and further investigation of intratumoral heterogeneity could provide additional biological insight into the role of *IGF2BP1* in human meningiomas. Nevertheless, these findings have clinical importance as *IGF2BP1* expression stratifies patient outcomes and may be utilized as a risk marker. Finally, small molecule inhibitors targeting *IGF2BP1* are currently being tested in pre-clinical settings [8, 15], and may represent promising therapeutics for high risk meningiomas, however, may be limited by systemic toxicity given *IGF2BP1* role's in normal tissues.

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Data availability Raw RNA-sequencing data from human meningioma samples (GSE183653, GSE212666) as well as IGF2BP1 eCLIP-seq (GSE92021) and FOXM1 ChIP-seq (GSM1010856, GSM1010769, GSM1010750, GSM1010731, GSE105901, GSE105524) data from ENCODE cell lines is publicly available on the Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo>). Spatial transcriptomic data from human meningioma samples (PRJNA950017) is publicly available in the Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>). Processed data for gene expression and associated analyses is available in Supplementary Table S1.

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References

- Brastianos PK, Galanis E, Butowski N, Chan JW, Dunn IF, Goldbrunner R et al (2019) Advances in multidisciplinary therapy for meningiomas. *Neuro Oncol* 21:i18–i31. <https://doi.org/10.1093/neuonc/nyy136>
- Chen WC, Choudhury A, Youngblood MW, Polley MC, Lucas CG, Mirchia K et al (2023) Targeted gene expression profiling predicts meningioma outcomes and radiotherapy responses. *Nat Med*. <https://doi.org/10.1038/s41591-023-02586-z>
- Choudhury A, Magill ST, Eaton CD, Prager BC, Chen WC, Cady MA et al (2022) Meningioma DNA methylation groups identify biological drivers and therapeutic vulnerabilities. *Nat Genet* 54:649–659. <https://doi.org/10.1038/s41588-022-01061-8>
- Kishida Y, Natsume A, Kondo Y, Takeuchi I, An B, Okamoto Y et al (2012) Epigenetic subclassification of meningiomas based on genome-wide DNA methylation analyses. *Carcinogenesis* 33:436–441. <https://doi.org/10.1093/carcin/bgr260>
- Lan Q, Liu PY, Haase J, Bell JL, Huttelmaier S, Liu T (2019) The critical role of RNA m(6)A methylation in cancer. *Cancer Res* 79:1285–1292. <https://doi.org/10.1158/0008-5472.CAN-18-2965>
- Leclair NK, Choudhury A, Chen WC, Magill ST, McCortney K, Horbinski CM, Chen Z, Goldschmidt E, Eaton CD, Bulsara KR, Linda Bi W, Patel AJ, Sahm F, Raleigh D, Anczukow O (2024) RNA splicing as a biomarker and phenotypic driver of meningioma DNA methylation groups. *Neuro-Oncology*. <https://doi.org/10.1093/neuonc/noae150>
- Lucas CG, Mirchia K, Seo K, Najem H, Chen WC, Zakimi N et al (2024) Spatial genomic, biochemical and cellular mechanisms underlying meningioma heterogeneity and evolution. *Nat Genet*. <https://doi.org/10.1038/s41588-024-01747-1>
- Muller S, Bley N, Busch B, Glass M, Lederer M, Misiak C et al (2020) The oncofetal RNA-binding protein IGF2BP1 is a drug-gable, post-transcriptional super-enhancer of E2F-driven gene expression in cancer. *Nucleic Acids Res* 48:8576–8590. <https://doi.org/10.1093/nar/gkaa653>
- Nassiri F, Liu J, Patil V, Mamatjan Y, Wang JZ, Hugh-White R et al (2021) A clinically applicable integrative molecular classification of meningiomas. *Nature* 597:119–125. <https://doi.org/10.1038/s41586-021-03850-3>
- Sahm F, Schrimpf D, Stichel D, Jones DTW, Hielscher T, Schefzyk S et al (2017) DNA methylation-based classification and grading system for meningioma: a multicentre, retrospective analysis. *Lancet Oncol* 18:682–694. [https://doi.org/10.1016/S1470-2045\(17\)30155-9](https://doi.org/10.1016/S1470-2045(17)30155-9)
- Shao W, Zhao H, Zhang S, Ding Q, Guo Y, Hou K et al (2022) A pan-cancer landscape of IGF2BPs and their association with prognosis, stemness and tumor immune microenvironment. *Front Oncol* 12:1049183. <https://doi.org/10.3389/fonc.2022.1049183>
- Van Nostrand EL, Freese P, Pratt GA, Wang X, Wei X, Xiao R et al (2020) A large-scale binding and functional map of human RNA-binding proteins. *Nature* 583:711–719. <https://doi.org/10.1038/s41586-020-2077-3>
- Vasudevan HN, Braunstein SE, Phillips JJ, Pekmezci M, Tomlin BA, Wu A et al (2018) Comprehensive molecular profiling identifies FOXM1 as a key transcription factor for meningioma proliferation. *Cell Rep* 22:3672–3683. <https://doi.org/10.1016/j.celrep.2018.03.013>
- Vengoechea J, Sloan AE, Chen Y, Guan X, Ostrom QT, Kerstetter A et al (2013) Methylation markers of malignant potential in meningiomas. *J Neurosurg* 119:899–906. <https://doi.org/10.3171/2013.7.JNS13311>
- Wallis N, Oberman F, Shurrush K, Germain N, Greenwald G, Gershon T et al (2022) Small molecule inhibitor of Igf2bp1 represses Kras and a pro-oncogenic phenotype in cancer cells. *RNA Biol* 19:26–43. <https://doi.org/10.1080/15476286.2021.2010983>

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