BACKGROUND: Melanoma has the highest tropism of any cancer to metastasize to the brain, and 40% of late-stage patients develop brain metastasis. Invasion, survival, and progression of tumors is dependent on the support of the surrounding microenvironment; therefore, modulation of neighboring cells is a key factor in metastasis. Extracellular vesicles (EVs) are important in cell-to-cell signalling, shuttling proteins, RNA and DNA to alter the surroundings into a favorable tumor microenvironment. Our aims were to investigate the role of melanoma brain metastasis (MBM) derived EVs in MBM development to find possible contributing mechanisms to cancer progression for eventual therapeutic targeting. MATERIAL AND METHODS: MBM-EVs isolated via sequential ultracentrifugation were injected into mice as a pre-treatment prior to intracardiac injection of MBM cells. EVs were co-cultured with normal human astrocytes (NHA) to investigate phenotype changes. miRNA sequencing was performed on EVs collected from MBM cells and compared to NHA and melanocytes to determine a candidate miRNA signature. In situ hybridization was utilized to evaluate the level of miRNA in clinical patient MBM samples. Functional in vivo validation was performed by injecting mRNA knockdown MBM cells into mice. Sequencing of NHA in the presence or absence of target miRNA mimics was used to determine downstream targets. RESULTS: EVs primed with EVs had a significant increase in MBM tumor burden, compared to non-primed mice. Co-culture with MBM-EVs resulted in NHA activation in vitro, with increased proliferation, invasion, cytokine production, and upregulation of GFAP. miR-146a was highly upregulated in MBM EVs, and miR-146a mimics activated NHA. Patient samples had a significant increase in miR-146a expression, compared to healthy brain controls. MiR-146a knockdown in MBM mice models reduced MBM tumor burden. A cell line containing a hypoxia-inducible animal model was determined NUMB, an inhibitor of the Notch signalling pathway, as a target of miR-146a. Numb and other downstream Notch proteins expression was significantly altered in NHA in the presence of both MBM-EVs and miR-146a. CONCLUSION: In conclusion, EVs are important regulators of MBM and establish tumor-supporting reactive astrocytes by delivery of miR-146a. MiR-146a alters Notch signalling in astrocytes via inhibition of the tumor suppressor gene NUMB. Elevated miR-146a levels in patients suggests a potential clinical intervention is possible via miR-146a targeting.

P12.10.A. SUPRATENTORIAL Glioblastoma with SPINAL METASTASIS: A CASE REPORT WITH MOLECULAR PROFILING R. Lencakelev1, M. Hendrych1, P. Solar1, M. Hermanova1, O. Slaby2; St. Anne’s University Hospital, Brno, Czech Republic, 2Medical Faculty, Masaryk University, Brno, Czech Republic.

BACKGROUND: Glioblastoma (GBM) is regarded as an aggressive brain tumor that rarely develops extracranial metastases. Despite well-investigated molecular alterations in GBM, there is a limited understanding of these associated with the metastatic potential of GBM. MATERIAL AND METHODS: We present a case report of a 43-year-old woman with frontal GBM IDH-wildtype, who underwent gross-total resection followed by chemoradiation. Five months after surgery, the patient was diagnosed with an intraspinal GBM metastasis. Next-generation sequencing analysis of both primary and metastatic GBM tissues was performed using the Illumina TruSight Tumor 170 assay. RESULTS: The number of single nucleotide variants observed in the metastatic sample was substantially higher. Mutations in TP53, PTEN, and ARID1A were observed in the primary and metastatic tissue samples are indicative of the mesenchymal GBM subtype. Among others, in the metastatic sample, there were detected two inactivating mutations (Arg1026Ile, Trp1831Ter) in the NF1 gene. Further, a novel NOTCH1 frameshift mutation and ARID1A copy number variation were identified. CONCLUSION: In conclusion, EVs are important regulators of MBM and establish tumor-supporting reactive astrocytes by delivery of miR-146a. MiR-146a alters Notch signalling in astrocytes via inhibition of the tumor suppressor gene NUMB. Elevated miR-146a levels in patients suggests a potential clinical intervention is possible via miR-146a targeting.

P12.11.B. THE ROLE OF MiRNAs IN GLIOMA IN RESPONSE TO HYPOXIA L. C. Smith1, D. Chakraborty2, D. Lourdsamy3, D. Mchtyre4, D. Smith1; 1University of Nottingham, Nottingham, United Kingdom, 2University of Dhaka, Dhaka, Bangladesh.

BACKGROUND: Hypoxia, low oxygen, is a microenvironment that promotes tumour progression, particularly in gliomas. The regulation of many biological processes are maintained by miRNAs. The hypoxia status of glioma cells effects the regulation of processes by affecting the expression of individual miRNAs. The aims of this study is identifying significant miRNAs expression changes in glioblastoma under hypoxic conditions in glioma cells and further exploring the effect of hypoxia on miR-92a-3p and miR-149-5p in gliomas on the apoptotic and cellular senescence pathways. MATERIAL AND METHODS: A range of glioma cells were used for screening including primary cell lines: GIN28 and GIN31; low-grade cell lines: LGG19 and LGG24; paediatric cell line: SF188 and commercially available glioblastoma cell line U87. These cells were culture at both 1% (hypoxia) and 20% (normoxia) oxygen levels. Screening of miRNAs was achieved by quantitative polymerase chain reaction (qPCR) using miRNA specific primers. Knockdown/mimics were achieved by transfecting with miR-92a-3p and miR-149-5p mimics and inhibitors. Caspase-glo assay was used to assess the effect of hypoxia on apoptosis. RESULTS: miR-92a-3p and miR-149-5p were found to be downregulated and upregulated respectively in response to hypoxia in glioma cells. Using primary cell line U87, significantly increased hypoxia-affected miRNAs in glioblastomas. This discovery will increase our knowledge and understanding of the hypoxia effect on miRNAs which may aid in direct targeted therapy to conquer hypoxia in glioblastomas.

P12.12.A. RESOLVING THE CELLULAR ARCHITECTURE OF INFILTRATION ZONES IN GLIOBLASTOMAS M. Ritter1,2, C. Blume3,4, A. Patel1,2, P. Sievers1,2, H. Dogan1,2, C. Herold-Mende5, W. Wick6,7, A. von Deimling6,8, F. Sahn1,2; 1Universitätsklinikum Heidelberg, Heidelberg, Germany, 2German Cancer Research Center (DKFZ), Heidelberg, Germany.

BACKGROUND: Therapy resistance and infiltration still pose major challenges in the treatment of glioma. In the tumour border niche, an interaction between different healthy cell types and malignant cells leads to therapy resistance, acquisition of stem-cell like features, and recurrence. However, studying the tumour border is quite challenging due to the lack of specific glioma markers. Although single cell datasets contain information about the abundance of different cell types, they still lack the spatial arrangement of primary tumor subtypes in the tumour border so far. However, one sample showed these problems, but are still limited by a low resolution or a limited number of detectable transcripts. MATERIAL AND METHODS: We applied the Vismu spatial transcriptomics platform on 18 FFPE and 11 fresh frozen gliomas to image the cellular architecture of the tumour border niche. In addition, the transcriptome of single nuclei isolated from the fresh frozen sections was also analysed. Using Seurat and a single cell reference set from Darmanis et al. (2017) the cell types were mapped onto the section and the expression of S100A9 and other molecular variation profiles (CND) was determined. RESULTS: Using a single cell reference set we were able to map different cell types onto different areas of the tumour border niche of gliomas. To control the mapping we selected a healthy marker used to detect healthy brain cells and CNV profile was used to identify the tumour. The mapping matched the expression of markers and the CNV profile and only showed minor differences. First analysis confirmed distinct distribution of oligodendrocytes and neurons at the tumour border. We could not detect an enrichment of tumour subtypes at the tumour border so far. However, we showed an enrichment of the mesenchymal subtype at the peritumoral region. CNVs also showed low intratumoral heterogeneity for most sections. CONCLUSION: Although the first results of the mapping of different cell types look promising, a larger reference set is necessary to fully understand the molecular architecture of the tumour border niche. Identification of hypoxia-affected miRNAs in glioblastomas. This discovery will increase our knowledge and understanding of the hypoxia effect on miRNAs which may aid in direct targeted therapy to conquer hypoxia in glioblastomas.