plasma proteins as genetic instruments and glioma data from the recent genome wide association study as the outcome (12,497 cases, 18,190 controls from six studies). To increase reliability of the instruments, we focused on 2,346 significant pQTLs (Bonferroni-corrected P threshold of $5x10^{-8}$) with no heterogeneity across studies and performed two-sample MR. For MR results that reached statistical significance, we further evaluated the findings by performing Steiger filtering to assess the directionality of effects (e.g. whether the proximal pQTL effect is on the protein which then influences glioma, or vice versa). All analyses were performed in the TwoSampleMR R package. RESULTS: The MR analysis identified three proteins strongly associated (1,398 tests, P threshold of $3.58x10^{-5}$) with glioma: CD36, SEMA6A and CDH5. The top hit, CD36, is known to play a part in angiogenesis and its expression has been found to affect prognosis for patients with GBM. These associations are necessary but not sufficient to prove causality, as potential horizontal pleiotropy remains an alternative explanation. CONCLUSION: The results provide three potential new targets for the prevention of GBM progression, provided the causes of incidence are the same as progression. Upon further research, this could be evidence that MR may be a useful tool in the drug discovery process and have translational uses.

P04.73 DETECTION OF PROTEIN MARKERS OF TWO IDH1 GENE MUTATION (R132H, R132S) BY IMMUNOHISTOCHEMISTRY IN BRAIN ASTROCYTOMAS

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BACKGROUND: Astrocytoma is the most common primary tumor of CNS. Genetic alternations in glioma have increasing importance for classification puroposes. One of these genetic pathways is IDH1 mutation which has potential roles in diagnosis, prognosis and response to treatments. Our aim was to investigate IDH1 R132H and IDH1 R132S mutations by immunohistochemistry in brain astrocytomas. MATERIAL AND METHODS: IDH1 R132H and IDH1 R132S mutations were assessed by immunohistochemistry in 80 glioma patients from different histologic grades(20 patients from each grade),which are diagnosed in Ghaem and musa ibn jafar hospitals in mashhad,between 1388 and 1395.Then the results were compared with different factors such as,sex,gender and pattern of immunopositivity(negative,focal and diffuse).data results were analyzed by SPSS statistics. RESULTS: In our study the mean age was 16.9 years old in grade I,40 years in grade II,33.3 years in grade III and 54.5 years in grade IV,which correlation is most likely to be statistically significant. (P value<0.05). The prevalence of R132H IDH1 mutation were the same in grades II and III 50%(10/20) and was 55%(11/20) in gardeIV,but no mutation was detected in gardeI (P value<0.0001). The R132S IDH1 mutation did not identify in grade I of glioma,but was 10% in grade II,35% in grade III and 40% in garde IV(P value<0.05). The R132H and R132S IDH1 mutations were identified in 40/80 (50%)of tumors.The prevalence of IDH1 mutation was 55%(11/20) in grade II,70%(14/20)in grade III and 75%(15/20) in grade IV,but no mutation was detected in grade I(P value<0.0001). There was no correlation between sex,gender and pattern of immunopositivity and prevalence of IDH1 mutation in each garde.(Pvalue>0.05). CONCLUSION: The prevalence of IDH1 mutation is increased from grade II to grade IV,which may indicate that high grade gliomas are arising from low grade tumors, and also shows that pilocytic astrocytoma (grade I) has a different genetic pathway. Using immunohistochemistry is an easy and fast way to identify the IDH1 mutation, it could be use as first line for diagnosis the mutation, although it requires to confirm for its sensitivity and specificity in feature investigations in iran.also further studies is needed to evaluate the prognostic role of IDH1 mutation in Iranian people.According to the importance of IDH1 mutation in survival of the patients,we suggest that pathologists, clinicians and oncologists in our country should include this mutation for their diagnostic and therapeutic planes.

P04.74 PRECLINICAL EVALUATION OF COMBINATIONS TARGETING THE DNA DAMAGE RESPONSE IN 2D AND 3D MODELS OF GLIOBLASTOMA STEM CELLS

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BACKGROUND: Despite surgical resection followed by DNA-damaging adjuvant therapies, glioblastoma remain incurable. Increasing evidence demonstrates that aberrations within the DNA damage response (DDR) of cancer stem cells contribute to treatment resistance. We have previously shown that the Fanconi Anaemia (FA) pathway, a key DDR process, remains inactive in normal brain but is re-activated in glioblastoma, making it an appealing foundational target for cancer-specific combination therapies. Since intratumoural heterogeneity in glioblastoma and inherent capacity for functional redundancy within DDR networks are established concepts - we aimed to determine whether combined and hypothesis-driven targeting of the FA pathway along with interconnected DDR processes could form a basis for effective multimodal therapies. MATERIAL AND METHODS: Bioinformatic analysis of mRNA expression data (REMBRANDT database) was used to confirm the relevance of FA pathway activity in glioma. Subsequently, immunofluorescence and cell viability assays were used to validate and establish the therapeutic potential of novel FA pathway inhibitors (nFAPi) and inhibition of related DDR targets in established cell models. Finally, combinations targeting the DDR were optimised using immunoblotting, and assessed using clonogenic survival in 2D and novel 3D patient-derived glioblastoma stem cell models. RESULTS: High expression of downstream FA pathway genes is strongly associated with poor survival (-17.1% 5-year OS, n=329, Log-rank, $P<0.0001$) and is a feature of more aggressive tumour biology (log2-relative expression, glioblastoma vs WHO Grade I-III gliomas: 7.18 ± 0.02 vs 7.08 ± 0.02, unpaired *t*-test, $P=0.0009$). Furthermore, FAPi and nFAPi were confirmed to attenuate key markers of FA pathway activity. Of translational importance, nFAPi sensitised glioblastoma cells to temozolomide (comparison of fits F value 63.7, $P<0.0001$). Inhibitors of PARP1 (PARPi) and ATR also demonstrated potential as temozolomide-sensitising agents. Furthermore, combined FAPi and PARPi significantly reduced cell viability (F value 13.0, $P<0.0001$) and enhanced radiosensitisation in G7 & G1 patient-derived 3D models with sensitiser enhancement ratios ($SER_{0.37}$) of 1.51 (1.50–1.51, 95% CI) and 1.57 (1.52–1.61), respectively. Quantification of α/β ratio enhancement suggests combined DDR targeting may fundamentally alter the response of glioblastoma stem cells to irradiation (IR) - ratios (G7): DMSO 2.5 (1.6–2.9), FAPi 6.4 (5.8–7.7), PARPi 7.4 (6.4–10.1), combination 23.1 (14.6–108.5). CONCLUSION: Simultaneously targeting the FA pathway and interconnected DDR processes in glioblastoma represents a promising therapeutic strategy. Early mechanistic studies suggest this approach augments DNA damage and enhances IR-induced cell cycle arrest in G2/M, however further preclinical evaluation is ongoing.

P04.75 TIBOLONE, AN ESTROGENIC PROGESTIN OF HORMONE REPLACEMENT THERAPY REDUCES COLONY FORMATION AND 3-DIMENSIONAL SPHEROID S-PHASE IN C6 RAT GLIOMA *IN VITRO*

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BACKGROUND: Risk of high-grade gliomas is lower in young females and its incidence enhances after menopause suggesting a likely protective role of female hormones. Hormone replacement therapy (HRT) is being employed to treat osteoporosis and some epidemiological studies showed that HRT containing progesterone analogs decrease the risk of glial tumors. Tibolone is a unique progesterone analog used in HRT with tissue-specific estrogenic effects. According to some studies, tibolone may enhance the risk of mammary and female reproductive cancers; but peculiarly, there exist several basic scientific data that it acts antitumoral at higher concentrations than which can be achieved in HRT. This feature resembles to another progesterone analog, medroxyprogesterone acetate (MPA). MPA enhances risk of breast cancer in HRT protocols; nonetheless, at high dosages, it can cause regression of far advanced breast, endometrium and renal cancers. Moreover, molecular studies have shown that MPA and tibolone induce very similar gene expression patterns. Since tibolone's pro-estrogenic effects occur particularly in bone and brain, we have hypothesized that high dose tibolone with both progestagenic and estrogenic actions may block glioma growth alone and/or in synergy with MPA or temozolomide. MATERIAL AND METHODS: Soft agar colony assay, 3H-thymidine test to determine the S-phase (DNA synthesis phase) and transmission electron microscopy were performed to determine the effects of tibolone on C6 rat glioma. RESULTS: Tibolone dose-dependently blocked soft agar colony growth of C6 glioma. Tibolone potentiated MPA-induced growth inhibition in soft agar colonies. Tibolone potentiated temozolomide's effect in reducing colony growth. Tibolone inhibited DNA synthesis in C6 rat glioma spheroids (as assessed by BrdU-labeling index) to similar levels which can be achieved with temozolomide. Transmission electron microscopical analyses revealed that mutual interactions between tibolone, MPA and temozolomide involve mitochondrial condensation, mitophagy and autophagy. CONCLUSION: Tibolone merits to be studied in further models of glioblastoma in vitro and in vivo.

P04.76 THE EXPRESSION OF CYTOTOXIC T-LYMPHOCYTE-ASSOCIATED ANTIGEN 4 (CTLA4) IS UPREGULATED IN HIGH GRADE GLIOMAS

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