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far. Therefore, the search for new therapies is necessary. In this paper, we hypothesized improving the complications of chronic ischemic stroke in induced Sprague-Dawley rat model by intraluminal suture middle cerebral artery occlusion (MCAo), utilizing the combination of cell therapy and gene therapy. A new version of astrocytes is proposed by making some changes in their genome. To gain this goal, a gene profile including IL-38 (the most modern anti-inflammatory agent, which barricades inflammatory response factors), BRAG-1 (an anti-apoptotic gene from BCL-2 family), IL-38 and BRAG-1's complementary scaffold RNAs for their expression by deadCas9 (dCas9), complementary scaffold RNAs of LZK and MST-1 for their deletion, and deadCas9 gene is used. We hypothesized using modified astrocytes by dCas9, which is the most accurate genome-editing technology with the least side effects, in improving the complications of chronic ischemic stroke.

Methods: Searching online on google scholar, PubMed, and Scopus based on the keywords including Chronic ischemic stroke, Gene therapy, CRISPR, Scaffold RNAs, and Cell therapy.

Results: The global occurrence level of stroke seemed to be steady between1990-2010, whereas there are some gain of 68, 84, 12, and 26%, in orderly in the occurrence of primary stroke, an outbreak of the stroke disability-adjusted lifespan lost, and the mortality rate of stroke. As mentioned before, stroke is the second cause of death universally, and there is no FDA-approved therapy and medication without any side effects for chronic ischemic stroke; for acute ischemic stroke, there is only one FDA-approved therapy, tissue plasminogen activator (tPA) with many side effects. So, research for a novel and safe therapy for chronic ischemic stroke is significant. In this hypothesis, we appraise the effects of transducing the gene profile into the ex vivo astrocytes from the brain of Sprague-Dawley rat models before induction of chronic ischemic stroke in them by Intraluminal suture middle cerebral artery occlusion (MCAo). The gene profile consists of IL-38 as an anti-inflammatory agent, BRAG-1 as an anti-apoptotic factor, IL-38, and BRAG-1's complementary scaffold RNAs for their expression by dCas9, complementary scaffold RNAs of LZK and MST-1 for their deletion, and deadCas9 gene. Leucine zipper-bearing kinase (LZK) has a crucial role in glial scar formation, while macrophage-stimulating 1 (MST-1) has a crucial role in the pathophysiological process of various neurological disorders and oxidative stress-induced neuronal cell decease. One problem in amending the deficits of chronic ischemic stroke is inflammation. Although astrocytes have an immunomodulatory function, IL-38 is transfected into ex vivo astrocytes to augment their anti-inflammatory action, which is the newest anti-inflammatory agent from the IL-1 family. It acts like IL-1 receptor antagonist (IL-1Ra) and IL-36Ra, which prevents the production of T-cell cytokines like IL-17, IL-22, and IL-8; so, it barricades inflammatory responses. LZK is a conserved mitogen-activated protein kinase kinase kinase (MAPKKK) upstream of c-Jun N-terminal kinase (JNK) in the mitogen-activated protein kinase (MAPK) pathway. It has been reported that the omission of LZK in adult mice astrocytes decreases astrogliosis and prevents scar formation in spinal cord injuries, so, by LZK deletion in astrocytes, their glial scar formation function could be inhibited. Also, based on the brain environment, astrocytes have different functions. Immediately after the stroke, they accumulate around the ischemic site to confine its propagation, repairing the blood-brain barrier, and confining the spread of inflammation and brain damage by producing a glial scar. Even though glial scar acts as a physical barrier and releases chondroitin sulfate proteoglycans (CSPGs), which prevents axon growth and regeneration, the recent pieces of evidence have demonstrated that glial scar helps in axon growth, but prevents the natural migration of NSCs. However, after stroke, astrocytes act differently. They have a significant role in fibrin devastation in CNS by preparing a surface for tissue plasminogen activator, which stimulates pro-brain-derived neurotrophic factor (pro-BDNF) and fibrinogen devastation, which prepares the ischemic area for the natural migration of NSCs. Another problem in the ischemic area is hypoxic death due to lack of circulation below 50%, which results in the primary decrease of migrant NSCs, injected cells, and their derived cells. For preventing this hurdle, BRAG-1 (an anti-apoptotic agent from the BCL2 family) is transduced to astrocytes and MST-1, necessary for oxidative stress-induced neuronal cell death, is inhibited. Moreover, pieces of evidence showed that specific deletion of MST-1 in microglia relieves stroke-induced brain injury. Besides, astrocytes have a crucial role in controlling circulation and angiogenesis. This simultaneous expression and inhibition of these genes are just available by the newest, the most precise, cost-effective, and the easiest genome editing tool deadCas9 and complementary scaffold RNAs of each gene. Another advantage of the CRISPR system is the possibility of switching the system off by anti-CRISPR proteins, whenever there are scarce side effects. As a result, a new version of astrocytes is produced, which their glia scar formation function is omitted, which makes the area ready for NSCs migration and following motor function improvement; they are resistant to hypoxic death and apoptosis; their anti-inflammatory role is amplified, and they have their angiogenesis function by releasing vascular endothelial growth factor (VEGF). Therefore, it is expected that they will be a novel multiplexed therapy of chronic ischemic stroke through a multiplex CRISPR system in SD rat models.

Conclusion: The new version of astrocytes is proposed, which their glia scar formation function is omitted, which makes the area ready for NSCs migration and following motor function improvement. Besides, they are resistant to hypoxic death and apoptosis, their anti-inflammatory function is amplified, and they have their angiogenesis function by releasing VEGF. So, this could be the novel multiplexed therapy of chronic ischemic stroke in SD rat models, which has the potential to be used in the clinic, if further evaluation is performed.

Keywords: Chronic ischemic stroke, Gene therapy, CRISPR, Scaffold RNAs, Cell therapy

O-23 Expression and function of C1orf132 long-noncoding RNA, in breast cancer cell lines and tissues

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Background and Aim: miR-29b2 and miR-29c play a suppressive role in breast cancer progression. Mir29b2chg/C1orf132 is the host gene for generating both microRNAs. However, there existed longer transcripts of the gene, with unknown function.
Methods: Here, we have employed bioinformatics and experimental approaches to decipher expression of two different C1orf132 transcripts in different types of breast cancer cell lines and tissues.
Results: Our data demonstrated a significant downregulation of C1orf132 in triple-negative type of breast cancer. We also find out that a mouse homolog of C1orf132 is located at a similar genomic location as its human counterpart, between CD46 and CD34 genes.
Conclusion: The latter finding suggests new potential functions for the lncRNA, other than being a simple miR-29 host gene. We predicted a putative promoter for the longer transcripts of C1orf132. The functionality of the distal promoter confirmed by transfecting MCF7 cells with a C1orf132 promoter-GFP construct. Knocking-down the promoter by means of the Crispr/Cas9 approach revealed no alteration in the expression level of neighboring genes, CD46 and CD34. However, the expression level of miR-29c was reduced by half, suggesting an enhancer effect of the distal promoter on miR-29cf generation. Furthermore, the promoter knock-down revealed a G2/M cell cycle arrest and an elevation of migration ability in Mcf12a edited cells. Moreover, the expression of cell mobility genes e.g. CDH2, FGF2, FGFR1 and the stem cell and EMT -associated transcription factor ZEB1 was significantly overexpressed in edited cells. Altogether, we are reporting here the existence of an additional/distal promoter with an enhancer effect on miR-29 generation and an inhibitory effect on cell proliferation and cell migration.
Keywords: Long-noncoding RNA, Triple-negative breast cancer, Crispr/Cas9, EMT

O-24 Relationship between expression levels of RPS6KB1 and GLI1 with DYRK1B during adipogenesis of ADSCs in the presence and absence of R102C mutation in DYRK1B gene

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Background and Aim: Metabolic syndrome is increasing as a worldwide health problem, including Iran. The missense mutation of the DYRK1B gene R102C in an Iranian population has been shown to be correlated to a rare autosomal-dominant form of the metabolic syndrome. Rate of adipogenesis is increased in the presence of the mutated gene which can be through the interactions with Shh pathway although the details of DYRK1B interactions with other components of adipogenic pathways is not completely understood. Gli1 is a transcription factor and is the final effector of Shh pathway. Gene RPS6KB1 encodes a kinase called S6K and plays a role at early stages of adipogenesis. C/EBPα and PPARγ are two major regulators of adipogenesis and their expression levels increased during this process. In the current study we assessed the expression levels of DYRK1B, GLI1, RPS6KB1, C/EBPα and PPARγ in the day1, 5 and 10 after adipogenesis induction .
Methods: The ADSCs were obtained from two groups of donors, affected with R102C mutation in DYRK1B gene and non-affected ones, characterized using flow cytometry. The obtained ADSCs were cultured and differentiated into adipocytes. Adipogenic differentiation was confirmed with oil Red o staining. After total RNA extraction and cDNA synthesis, the gene expression levels were evaluated by Real-time PCR. The results were analyzed by two-way ANOVA test using Graph Pad Prism.
Results: After adipogenesis induction the expression of C/EBPα and PPARγ in both groups increased, also they were higher in the mutated group significantly. DYRK1B expression increased during the day1 and 5 and decreased at day10, not analytically significant. RPS6KB1 increased in the day1 and 5 and decreased at day10; GLI1 expression decreased in all days. Although Changes of RPS6KB1 and GLI1 were significant within each group, it wasn't between the groups.
Conclusion: The R102C mutation of DYRK1B can increase adipogenesis. During adipogenesis, Shh pathway activity is decreased (according to GLI1 descending levels). RPS6KB1 ascending levels in day1 and 5 is correlated with early stages of adipogenesis. Since the changes of RPS6KB1 and GLI1 aren't significant between groups, different rates of adipogenesis in the case R102C mutation cannot be related to GLI1 or S6K directly.
Keywords: Metabolic Syndrome, adipogenesis, DYK1B, GLI1, RPS6KB1 ,S6K

O-25 Investigating the effects of miR-138 replacement on inhibiting cell migration and induction of apoptosis in breast cancer cell line

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Background and Aim: MicroRNAs (small noncoding RNAs of 20-24 nucleotides long) are involved in the regulation of post-transcriptional gene expression, have close links with various cancers. Of these, miR-138 is a well-known microRNA with multiple tumor suppressor effects. In this study we investigated the effects of miR-138 replacement on inhibiting cell migration and induction of apoptosis in breast cancer cell line.
Methods: At first miR-138 mimic was transfected into MAD-MB-231 cells using electroporation method and optimum dose of miR-138 was determinded by MTT assay. At next stages we evaluated the effects of miR-138 mimic on cell migration and apoptosis using wound healing assay and Annexin V- FITC/PI kit (flowcytometry) respectively. All data was analyzed by