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The Mechanism Of LIL combined with AZD8055 On The Glutamine Addiction Of CT26 Cells

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Objective The photobiomodulation of low-intensity lasers can activate many pathways and molecules, which involved in tumor growth, invasion, metastasis, and resistance to drugs or radiation therapy, but studies of low-intensity lasers on tumor cell addiction, have not been reported. Numerous studies have shown that changes in plasma and muscle glutamine levels can occur when the body undergoes prolonged exercise (>1 hour) or overtraining. AZD8055 is a dual inhibitor of mammalian rapamycin complex (mTORC)1/mTORC2 that inhibits phosphorylation of mTORC1 (p70S6K and 4E-BP1), mTORC2 (AKT) and downstream proteins.
Based on the experimental model of glutamine addiction in laboratory, we chose the worst conditions to cause super-proliferation of CT26 cells and simulate the drug resistance of solid tumors. The purpose is to study tumor deterioration from the perspective of tumor cell function. Under the state, the effect of low-intensity laser combined with AZD8055 on the proliferation of glutamine addiction in CT26 cells is also the development of exercise intervention tumors, and the experimental basis is proposed from the perspective of nutrient metabolism.
Methods This experiment used a pre-experimental glutamine addiction model to culture mouse colon cancer CT26 cells after horse serum shock, and used different intensity 640±15 nm low-intensity lasers from light-emitting diode arrays (red light at 640±15 nm from Light emitting diode array, RLED), 15 min/day； different concentrations of AZD8055 (0 ~ 100μM) on different proliferative CT26 cells at different times.
Cell proliferation was detected by MTT assay; mRNA levels of proliferation-related genes were detected by RT-PCR; protein levels were detected by western blot.
The data were analyzed by SPSS21.0 statistical software and self-similarity index analysis. Among them, the statistical significance level was set at 0.05, $P<0.05$ was different; $P<0.01$ was significant difference. In the self-similarity index analysis method, $l>0.8$ is a significant difference.
Results 1.CT26 cells were incubated at Gln7.94 mM until the 8th day, and AZD8055 was added at different concentrations. Compared with no inhibitor , and at 6 h, the cells were significantly increased when the concentration of AZD8055 was 0.001 μM, 1 μM, 10 μM and 100 μM. ($P<0.01$); at 12h, the cells were significantly inhibition when the of AZD8055>0.5μM ($P<0.01$).
2.From the growth inhibition rate analysis, at 48 h and 72 h, the inhibition rate exceeded 50%,when the concentration of AZD8055 was 100 μM (61.01% and 87.46%), when< 0.1 μM, the cells were proliferation occurred at 48 h and 72 h, and it is drug resistance.
3.When AZD8055 combined with low-intensity laser treatment of CT26 cells, CT26 cells had different degrees of proliferative effects at different time points: 12h, 24h, 48h and 72h. Especially at 48h, the light intensity of LIL2 to LIL8 had a significant proliferative effect on CT26 cells ($l\geq0.8$).
4.Compared with the control group (0 hours without inhibitor), the CT26 cells were treatment with AZD8055 1μM , the mRNA level of the gene GLUT1 was significantly down-regulated in all time periods ($P<0.01$). When the LIL(2.17 mW/cm^2) combined AZD8055 (1μM) significantly promoted the proliferation of CT26 cells at 24h and 48h. It is related to up-regulated of protein expression in all time points: at 6h and 12h, the pMEK protein and the pMEK/ MEK were significantly up-regulated($P<0.01$). At 24h, the expression of GLUT1 protein was up-regulated($P<0.01$), MEK protein,

pERK protein and ERK/ pERK were significantly up-regulated (P<0.01). At 48h, the GLUT1 protein, MEK protein , pERK protein and pERK/ERK were up-regulated (P<0.01).
Conclusions 1.The minimum inhibitory concentration of AZD8055 on super-proliferative CT26 cells was 0.5 μM, and the super-proliferative state showed more obvious drug resistance.
2.When low-intensity lasers combined with AZD8055 treated CT26 super-proliferating cells, CT26 cells are not sensitive to AZD8055, while low-intensity lasers promote CT26 cells proliferation by up-regulating MEK/ERK signaling pathways.