Abstract

Oral cancer is the sixth leading cancer in the world. Cisplatin is the first-line of chemotherapeutic drug for the treatment of oral cancer, however, cancer with cisplatin resistance is one of the major cause of treatment failure. To investigate the mechanism of cisplatin resistance, we established cisplatin-resistant (CR) sublines from SAS, CGHNC8, CGHNC9, and OE0-M1 oral cancer cell lines. Global survey by transcriptomic profiling analysis was performed between parental and CR subline and identified 16 cisplatin-resistant candidate genes; and confirmed by RT-QPCR or western blotting. The average mRNA and protein expression level of KCNJ2 is the obvious in CR subline cells. Thus, KCNJ2 was selected for further investigation. KCNJ2 is a molecular potassium channel, which allows potassium flowing into cells to prevent cell apoptosis. To determine potential functions of KCNJ2 in cisplatin resistance, we have established KCNJ2 stable knocked down cells. The clonogenic assay showed that KCNJ2 stable knocked down cells were more sensitive to cisplatin compare to parental cells. Furthermore, intracellular potassium, which was stained by PBFI-AM, lost more and faster in KCNJ2 stable knocked down cells by using flow cytometry detection. On the other hand, stable silencing of KCNJ2 could induce both apoptotic protein expression earlier, and processed cell death. These results indicate that silencing of KCNJ2 could promote HNC cells more sensitive to cisplatin by losing intracellular potassium; and finally, induce cell apoptosis.

Results

Figure 1. Cell Viability of Cisplatin-Resistant (CR) Subline Cells of SAS, CGHNC8 and CGHNC9 is Higher Than of Parental cells. Cells were treated by various concentration of cisplatin (0-10 μg/mL) for 48 hours, and cell viability was detected by MTS assay. Untreated data was used as 100% survival rate.

Figure 2. Global Survey of Cisplatin-Resistant Candidate Genes by Affymetrix U133A Human cDNA Microarray. (A) Hierarchical cluster analysis of parental and cisplatin-resistant subline cells. The upper panel was the parental cells and the lower panel was the cisplatin-resistant subline cells. Red color represent up-regulated and blue as down-regulated. (B) Prediction of function network pathways associated with cisplatin resistance by Partek software analysis.

Figure 3. Verification of Cisplatin-Resistant Candidate Genes. (A) RT-QPCR was used to verify 16 candidate genes, which were selected by literature search. (B) The data of verification was integrated and plotted to form a graph of which p-value verse average fold change curve.

Figure 4. Protein Expression of KCNJ2 in CR Subline Cells Was Higher Than Parental Cells.

Figure 5. KCNJ2 stable knocked down cells are more sensitive to cisplatin treatment. 800 cells of (A) SAS and (B) C8 were seeded in 96-well plate, and treated with various concentration of cisplatin (0.1-1 μg/mL) for 24 hours. After incubating with drug-free medium for 7-10 days, cells were stained by crystal violet. The survival rate was quantified by colony counting and the knockdown efficiency was verified by western blotting.

Figure 6. Silencing of KCNJ2 lost intracellular potassium more and faster after cisplatin treatment. SAS or C8 Cells were treated with 5μg/mL of cisplatin for 0-72 hours. 1x10^4 cells were processed for flow cytometry analysis every 12 hour until 48hr. The fluorescence intensity were detected by flow cytometry. Populations of cells with differences in PBFI fluorescence represent the changes of intracellular potassium.

Figure 7. Silencing of KCNJ2 induced apoptotic caspases activation earlier. Caspases activity in control and KCNJ2 knockdown cells after cisplatin treatment were confirmed by western blot.

Figure 8. Silencing of KCNJ2 increased cell death after cisplatin treatment. Cells were treated with cisplatin and cell status was determined by Annexin V/PI staining and flow cytometry analysis every 12 hour until 48hr.

Conclusion

1. Cisplatin-resistant subline cells are more resistant to cisplatin than parental cells.
2. 345 candidate genes were up-regulated by transcriptomic analysis, 16 candidate genes were confirmed by RT-QPCR and KCNJ2 was selected to further investigation.
3. Stable knockdown of KCNJ2 would increase cells more sensitive to cisplatin treatment.
4. Stable knockdown of KCNJ2 would promote lose of intracellular potassium after cisplatin treatment.
5. Silencing of KCNJ2 would induce apoptotic protein express earlier after cisplatin treatment.

[Diagram showing the mechanism of how cisplatin affects cell death and the role of KCNJ2 in this process]