AB179. KCNJ1 inhibits tumor proliferation and metastasis and is a prognostic factor in clear cell renal cell carcinoma

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Objective: Potassium inwardly-rectifying channel, subfamily J, member 1 (KCNJ1), plays an essential role in potassium balance by transporting potassium out of cells. KCNJ1 variation is associated with multiple diseases, such as antenatal Bartter syndrome and diabetes. However, the role of KCNJ1 in clear cell renal cell carcinoma (ccRCC) is still unknown. The aim of this study was to detect the expression of KCNJ1 in ccRCC and determine the relationship of KCNJ1 expression with the progression and prognosis of ccRCC.

Methods: Expression of KCNJ1 was evaluated in ccRCC tissues and cell lines by qRT-PCR, Western blot and immunohistochemistry analysis. The relationship between KCNJ1 expression and clinicopathological characteristics was analyzed. P3xFLAG-CMV-14 vector containing KCNJ1 was constructed, and used for infecting ccRCC cell lines 786-O and Caki-2. The effects of KCNJ1 on cell proliferation, invasion and apoptosis were detected in ccRCC cell lines using cell proliferation assay, transwell assay and flow cytometry.

Results: KCNJ1 was low-expressed in ccRCC tissues samples and cell lines. The KCNJ1 expression level was significantly associated with tumor pathology grade (P=0.002), and clinical stage (P=0.023). Furthermore, the KCNJ1 expression was a prognostic factor of ccRCC patient's survival (P=0.033). The re-expression of KCNJ1 in 786-O and Caki-2 significantly inhibited cancer cell growth and invasion, and promoted cancer cell apoptosis.

Conclusions: Taken together, we concluded that KCNJ1, low-expressed in ccRCC and associated with poor prognosis, plays an important role in ccRCC cell growth and metastasis.

Keywords: KCNJ1; clear cell renal cell carcinoma (ccRCC); proliferation and metastasis; prognostic factor

doi: 10.3978/j.issn.2223-4683.2014.s179

Cite this abstract as: Guo Z, Liu J, Su B, Li X, Zhou L. KCNJ1 inhibits tumor proliferation and metastasis and is a prognostic factor in clear cell renal cell carcinoma. Transl Androl Urol 2014;3(S1):AB179. doi: 10.3978/j.issn.2223-4683.2014.s179

AB180. The charateristics and therapeutic applications of low-intensity pulsed ultrasound

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Abstract: Ultrasound is a form of mechanical energy with its acoustic pressure wave at frequencies range from 20 to 20,000 Hz. To date, ultrasound waves are not only used in imaging medicine for diagnosis, but also are performed in physical therapy (PT) medicine for the purpose of preventing and curing disease due to its thermal and non-thermal effects, and the ultrasound frequencies used in PT are typically between 1.0 and 3.0 MHz. Low-intensity pulsed ultrasound (LIPUS) typically has an intensity at 30 mW/cm, pulse ratio 1:4 at 1,000 Hz, and frequency at 1.5 MHz, which has been demonstrated to have lots of beneficial effects in promoting bone-fracture healing, accelerating soft-tissue healing, inhibiting inflammatory responses and so on. The underlying mechanisms of biological effects of therapeutic ultrasound in PT medicine may be associated with the upregulation of cell proliferation

through activation of integrin receptors and Rho/ROCK/Src/ERK signaling pathway, and with promoting multilineage differentiation of mesenchyme stem/progenitor cell lines through ROCK-Cot/Tpl2-MEK-ERK signaling pathway. However, it still needs an intense effort for basic-science and clinical investigators to explore the cellular and molecular mechanisms and biomedical applications of LIPUS on human body in the future.

Keywords: Low-intensity pulsed ultrasound (LIPUS); therapeutic ultrasound; ultrasound treatment

doi: 10.3978/j.issn.2223-4683.2014.s180

Cite this abstract as: Lei H, Xu Y, Guan R, Li H, Tian W, Wang L, Gao Z, Xin Z. The charateristics and therapeutic applications of low-intensity pulsed ultrasound. Transl Androl Urol 2014;3(S1):AB180. doi: 10.3978/j.issn.2223-4683.2014.s180

AB181. *In vitro* study on shRNA decreasing the expression of human testis-specific gene *TDRG1* and affecting the proliferation, invasion and apoptosis of NTERA-2 Cell

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Objective: We have screened and cloned a novel human testis-specific gene, named Testis developmental related gene 1 (*TDRG1* GenBank DQ168992), using Digital Differential Display. In our previous work, we hypothesized that this gene might be involved in the occurrence and development of testiculoma. To investigate this possibility, we have successfully established a stabilized RNA interference system to decrease the expression of *TDRG1* in NTERA-2 cell line. In this study, to explore the function of *TDRG1* in testiculoma further, we successfully transfected the *TDRG1*-shRNA486 and *TDRG1*-shRNA/control expression vectors described in our previous work (PubMed PMID: 23117448) into NTERA-2 cells *in vitro* and tested the biological behaviors of NTERA-2 cells.

Methods: Firstly, we checked the expression of *TDRG1* in NTERA-2 cell line. Then we used fluorescence microscope to ensure the successful transfection. The expression of *TDRG1* mRNA and protein was verified by fluorescence quantitative PCR and indirect cell immunofluorescence, respectively. Furthermore, we used such methods as MTT assay, transwell assay and flow cytometry to detect the biological behaviors of NTERA-2 cells.

Results: We determined that the *TDRG1* mRNA and protein levels were significantly reduced by TDRG1-shRNA486expression vector. Moreover, the ability to proliferate and invade *in vitro* was suppressed in cells where the expression of TDRG1 was down-regulated, and a corresponding increase in the apoptotic potential was observed.

Conclusions: Firstly, these results indicate that the *TDRG1*-shRNA486 expression vector constructed previously can interfere the *TDRG1* expression effectively and stably. We construct a good cell line model in which *TDRG1* expression was inhibited. Secondly, the ability of proliferation and invasion of NTERA-2 cells in vitro can be positively regulated by *TDRG1*, while the potential of apoptosis can be negatively regulated. This gene has some characters resembling oncogene.

Keywords: *TDRG1*; NTERA-2; siRNA interfering; cell biological; behavior

doi: 10.3978/j.issn.2223-4683.2014.s181

Cite this abstract as: Gan Y, Tang Y. *In vitro* study on shRNAdecreasing the expression of human testis-specific gene TDRG1 and affecting the proliferation, invasion and apoptosis of NTERA-2 Cell. Transl Androl Urol 2014;3(S1):AB181. doi: 10.3978/j.issn.2223-4683.2014.s181

