Abstract

**Decreased tumor progression by the knockdown of interleukin 17 receptor A in the B16F10 melanoma model**

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**Abstract**

The chronic inflammatory could increase the risk of developing many type of cancer. Therefore, inflammation is now known to contribute to the formation of tumor microenvironment, promoting proliferation, migration and survival of cancer cells that result in tumor cell invasion, migration and metastasis. The tumor cells also produce cytokines and chemokines for attracting immune cells to promote cancer development.

**Results:** As compared with those in the control B16F10 cells, the ymml-17 inducing cell proliferation and invasion were significantly reduced in the derived IL-17A-deficient cells. The tumor growth and metastasis were also apparently reduced in the mice bearing the IL-17A-deficient tumors. In fact, not only the expression of MMP2 and MMP9 but also the production of VEGF and HGF were reduced both in vitro and in vivo. Additionally, the increased levels of IFNγ and Granzyme B+ immune cells as well as the reduced numbers of Tregs and MDSCs appeared in the mice bearing the IL-17A deficient tumors, indicating the lack of IL-17 enhancing the CTL activity and other anti-tumor immunity.

**Conclusions:** These findings support the hypothesis that IL-17/IL-17RA signaling directly promotes tumor growth as well as recruits MDSCs and Treg cells, resulting in tumor progression. It suggests IL-17RA on tumors as a potential therapeutic target for cancer therapy.

**Introduction**

The lower chamber contained complete culture medium, which included 10% FBS and 1% penicillin/streptomycin, and was homogenized with bead by Qiagen TRIzol reagent following the manufacturer’s instructions, and one microgram of RNA was used for RT. cDNA synthesis was performed using SuperScript II (Invitrogen) to prepare targets by VEGF, HGF, MMP2, MMP9 primer, and β-actin as control. Total RNA of tumor tissue was homogenized with bead by TissueLyser (Qiagen), and also extracted with the TRIzol reagent.

**Materials and Methods**

Establishment of B16-f1 melanoma-bearing model

Adult male B6 mice with an average age of 8 weeks were utilized in the current study. A melanoma-bearing model was established by subcutaneous injection at central back with 100μl B16-F10 cells (3x10⁶/100μl) for the experimental groups.

**Bioimunascence imaging with IVIS**

Mice were anesthetized with isoflurane inhalation, and were subsequently intraperitoneally (i.p.) injected with 150μg/g D-luciferin (Xenogen). Bioimunascence imaging with a CCD camera (IVIS, Xenogen) was initiated 5 min after injection. The expression of transgene and metastases was measured at day 7, 14 and 21 following cell inoculation.

**ELISA**

B16F10 cells (5x10⁶/ml) were cultured with or without 100 ng/ml ymml-17 for 48h. Cell-free supernatants were collected. And the serum were collected from B16F10 tumor bearing mice. Concentrations of VEGF were measured using commercially available ELISA kits (R&D).

**RNA extraction and real time RT-PCR**

B16F10 were treated with ymml-17 (100 ng/ml, for 24 hours in vitro). Total RNA was extracted with the TRIzol reagent following the manufacturer’s instructions and instructions.

**Cell Migration and invasion assay**

After transfection with either shIL17RA or shCTRL plasmid, 2x10⁴ transfecants were seeded in 6 well dish and then incubated in the presence of 1% FBS culture medium for 48 h. A cell invasion assay was done by using a matrigel coating chamber. The cells transfected with shIL17RA or mock in 1% FBS medium were seeded into the upper chamber. The lower chamber contained complete culture medium, which included 10% FBS. Cells in the lower chamber, which have passed through the matrigel-coated membrane, were counted.

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**Summary**

• Blockade of IL-17A abolished tumor development, angiogenesis and metastasis in tumor tissues.
• Such strategy also improved anti-tumor immunity by enhancing CTL activity and suppressing MDSCs and Treg cells.

**References**


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