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学位論文題目	in vivo antitumor function of tumor antigen-specific CTLs generated in the presence of OX40 co-stimulation in vitro （in vitro で OX40 補助刺激存在下に誘導された腫瘍抗原特異的 CTL の in vivo での抗腫瘍機能について）
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別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

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学位論文題目	***In vivo* antitumor function of tumor antigen-specific CTLs generated in the presence of OX40 co-stimulation *in vitro*** （*in vitro* で OX40 補助刺激存在下に誘導された腫瘍抗原特異的 CTL の *in vivo* での抗腫瘍機能について）		

Purpose:

Adoptive cell transfer (ACT) is one of anticancer immunotherapies. In ACT, tumor-reactive T cells are generated to a large population before transfer back to patients with cancer. OX40, a member of TNRF (tumor necrosis factor receptor) family, are receptors expressed on activated $CD4^+$ and $CD8^+$ T cells. Antitumor effects of OX40 were well established when additional OX40 signals were induced in recipients before ACT. Here, we aim to investigate the affects of OX40 co-stimulation on the T cells generated *in vitro* as well as the *in vivo* antitumor effects of these OX40 co-stimulated T cells in afterward ACT.

Methods:

- **Tumor antigen**: We used wild type FVB mice in a model of mammary tumor. The tumor expressing neu antigen was established by NT cell line. Neu-expressing vaccine cell line 3T3 neu/GM was used to amplify neu-reactive T cells in donor mice. In vitro, neu-specific $CD8^+$T cells were activated and re-stimulated by RNEU peptide, an immunodominant peptide of neu, which was presented by an MHC I restricted APC cell: the $T2D^q$ cell line.
- ***In vitro* CTL generation**: Donor FVB mice had been inoculated with NT cell line and vaccinated with 3T3 neu/GM cell line before their splenic T cells were purified and cultivated for 7 days. The cultivation included T cells and RNEU-pulsed $T2D^q$ cells in the presence or absence of anti OX40 mAb. After 7 days, $CD8^+$ T cells were separated from cultivation by magnet field and were used in ACT.
- **Adoptive cell transfer therapy**: Recipient FVB mice were challenged with tumor NT cell line 3 days before receiving the $CD8^+$ T cells that had been co-stimulated by anti OX40 mAb or not. Control group was injected with PBS.
- **Evaluation methods**: Tumor sizes in recipients were observed every 3 days. Frequencies of neu-specific CTLs were assessed by ICS for the $CD8^+$ T cells secreting IFN-γ^+ as response to RNEU re-stimulation.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

Expressions of CD27, CD62L, CCR7, and Bcl2 were identified by ICS for corresponding marker gated on neu-specific CTLs population. Proliferation of neu-specific CTLs were adjusted by staining donor T cells before *in vitro* generation, otherwise staining the $CD8^+$ T cells before transfer with CFSE. The CFSE dilution gated on IFN-γ^+ $CD8^+$ T cells revealed proliferation profile of the cells.

Results:

The antigen-specific CTLs generated *in vitro* under OX40 co-stimulation proliferated less and therefore, expand to a smaller number than those generated without OX40 signal. However, the OX40 co-stimulated CTLs population expressed more CD27, CD62L, CCR7 markers, as well as Bcl2 protein than the CTLs that had not been co-stimulated *in vitro*.

After transfer to tumor-bearing recipients, CTLs derived from cultivation with anti OX40 mAb proliferated remarkably *in vivo*. They manifested potent antitumor function and finally eradicated the established tumors. Whereas, the CTLs not receiving OX40 signals *in vitro*, though being transferred with larger number, did not proliferated *in vivo* and just caused a transient delay in tumor growth. On day 45 after ACT, the transferred T cells that had been generated with OX40 signal were still detectable in recipient mice.

Discussion:

In vitro OX40 signal drove the generated CTLs towards earlier differentiation as they expressed more markers of early states like CD27, CD62L, CCR7. The younger CTLs possess higher proliferation potential and more effective antitumor function. OX40-derived CTLs also had more anti-apoptotic Bcl2 protein, which can explain for their long persistence *in vivo*.

Our findings established for the first time the benefits of OX40 signal *in vitro* towards the *in vivo* antitumor effects of CTLs generated for ACT. By appropriate modulation, a small number of CTLs could function effectively and finally eliminated tumor. This result consolidated the fact that quality of transferred cells is important contribution to successful ACT therapy.

Additional OX40 co-stimulation was induced in patients with progressive cancer as a signal modulation therapy. The trial showed ability in clinical application of OX40, but also quoted problem of systemic toxicity caused by the modulation molecule. By applying OX40 signal directly to T cells during *in vitro* expansion, the possibility of systemic toxics can be excluded while favorable affects of OX40 co-stimulation on tumor-reactive CTLs still remain.

Conclusions:

Generating CTLs with OX40 co-stimulation *in vitro* optimized the *in vivo* antitumor effects of these cells as ACT therapy. This approach prevents systemic toxicity in patients and minimizes the amount of expensive modulatory molecules.

別紙様式８（課程博士・論文博士共用）

学位論文審査の結果の要旨

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（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

本論文では、OX40 補助刺激下に *in vitro* で作製した抗原特異的 CTL を用いた養子免疫療法のマウス乳癌（HER2/neu 陽性）モデルにおける抗腫瘍効果を明らかにすることを目的として研究を行った。そのため、neu 発現 NT 腫瘍細胞を移植したドナーFVB マウスを neu 発現細胞である 3T3 neu/GM で免疫し、摘出した脾臓から採取した neu 反応性 T 細胞を RNEU ペプチドでパルスした $T2D^{q}$ 抗原提示細胞と共培養して neu 特異的 $CD8^{+}$ T 細胞（CTL）を作製した。NT 細胞移植 FVB マウスへ養子免疫する CTL は抗 OX40 抗体による補助刺激の有無で 2 通り用意し、PBS 群を加えた 3 群による投与試験を行い、以下の点を明らかにした。

１）OX40 補助刺激下では *in vitro* で作製される抗原特異的 CTL の数は少ない傾向にあるが、これらの CTL は CD27、CD62L、CCR7、Bcl2 を高レベルに発現している。

２）OX40 補助刺激下で作製された抗原特異的 CTL は *in vivo* で顕著に増殖し、抗腫瘍効果を示す。

本論文は、*in vitro* で OX40 補助刺激下に作製した CTL を用いた養子免疫療法のマウス乳癌モデルにおける有用性の一端を明らかとし、その応用に向け新たな知見を与えたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

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（平成３０年１月３０日）