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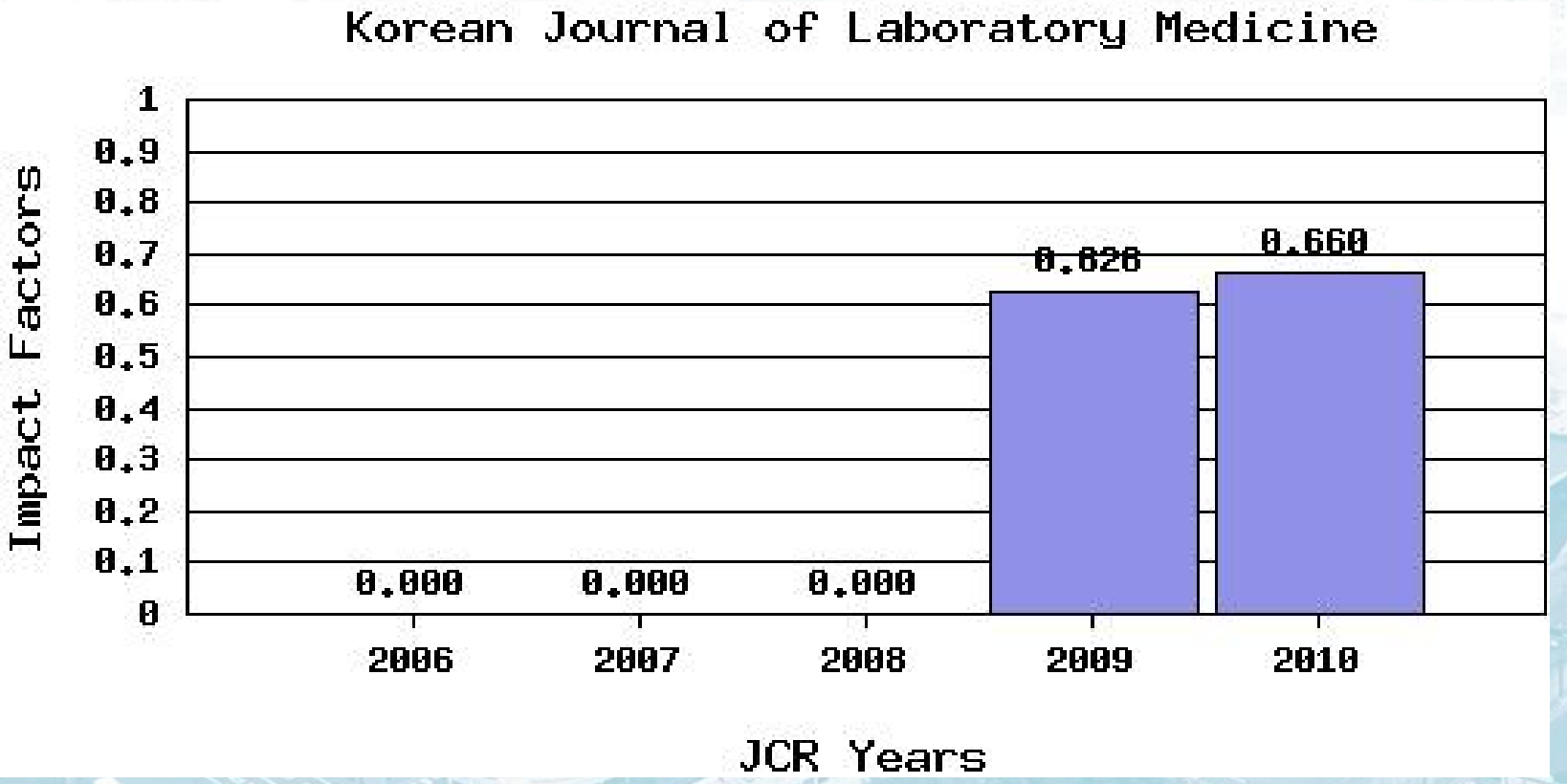

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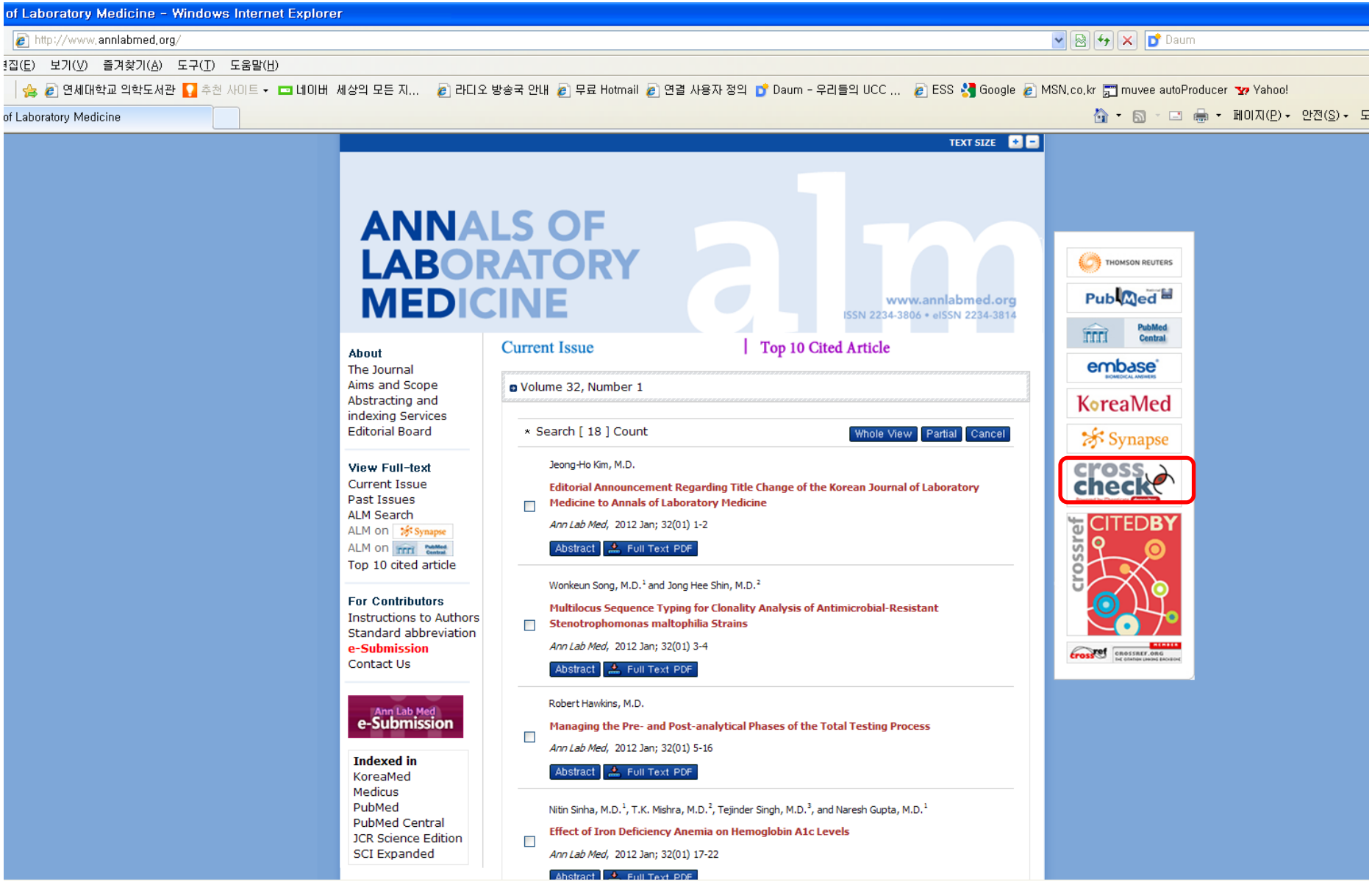

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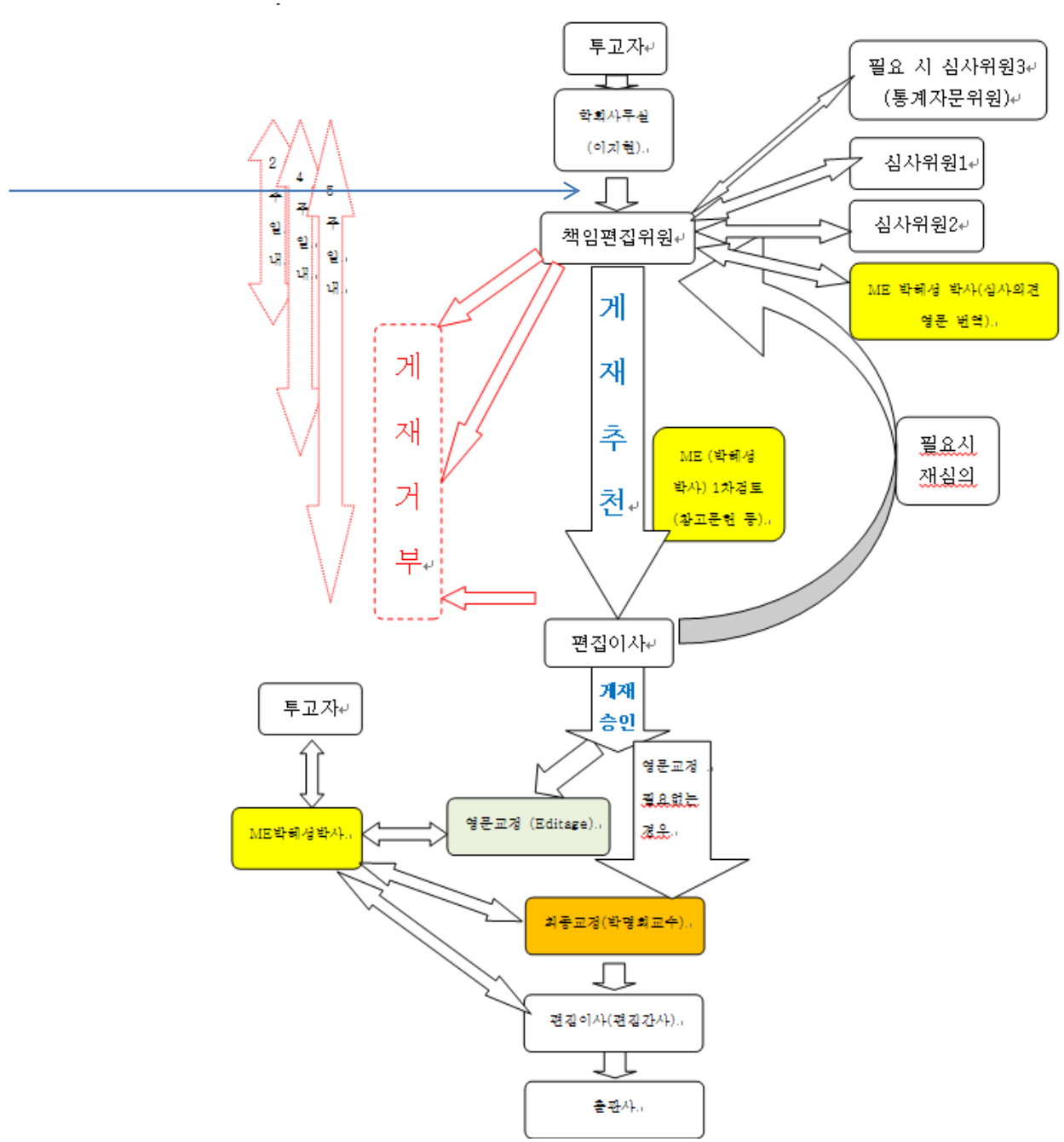

# A Case History

# Dear editor,

- We submit our manuscript entitled "4-1BB signaling on the biological function of murine dendritic cells" to The Korean Journal of Laboratory Medicine for publication.
- All authors (Kuang Youlin, Zhang Jianwei, Weng Xiaodong, Liu Xiuheng, Zhu Hengchen, Chen Zhiyuan) have seen the manuscript and approved to submit to your journal.
- This paper is an original article. Neither the entire paper nor any part of its content has been published or has been accepted elsewhere. It is not being submitted to any other journal.


- Corresponding authors**:** Liu Xiuheng, PhD, Department of Urology, Renmin Hospital of Wuhan University, Jiefang Road 238, Wuhan 430060, China.
- Tel.: +86 27 88041911-2235;
- fax: +86 27 88042292;
- e-mail: lxh670@163.com

-
- Thank you very much for your consideration.
- Kind regards.
- Yours sincerely,
-
- Liu Xiuheng

# A Journal Submission History

- KJLM-11-085 (Temp. No. 11111)
  - Journal Submission to the Korean J Lab Med
    - Jul 21-2011
  - Rejection by Editorial Decision by Associate Editor (Prof. KA Lee)
    - Jul 22-2011
    - Your manuscript has been examined by the editors.We concern that there is a significant level of similarity (text and data) to previously published works, especially YMJ 51:594,2010, without an appropriate attribution to the source.

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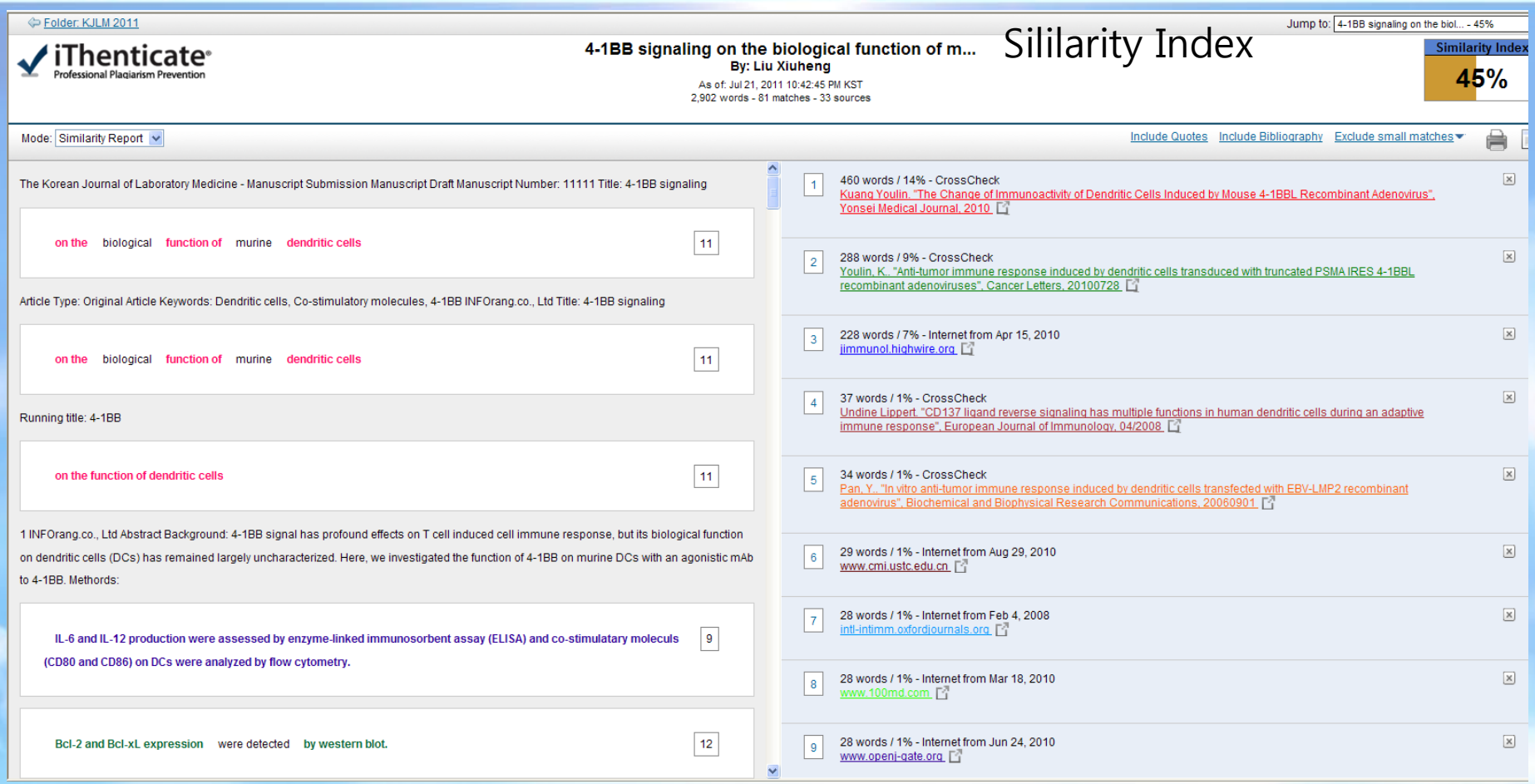

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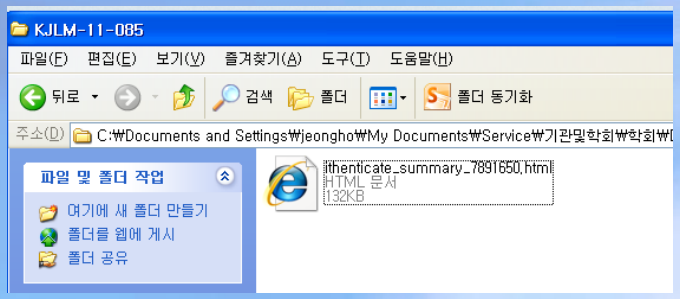


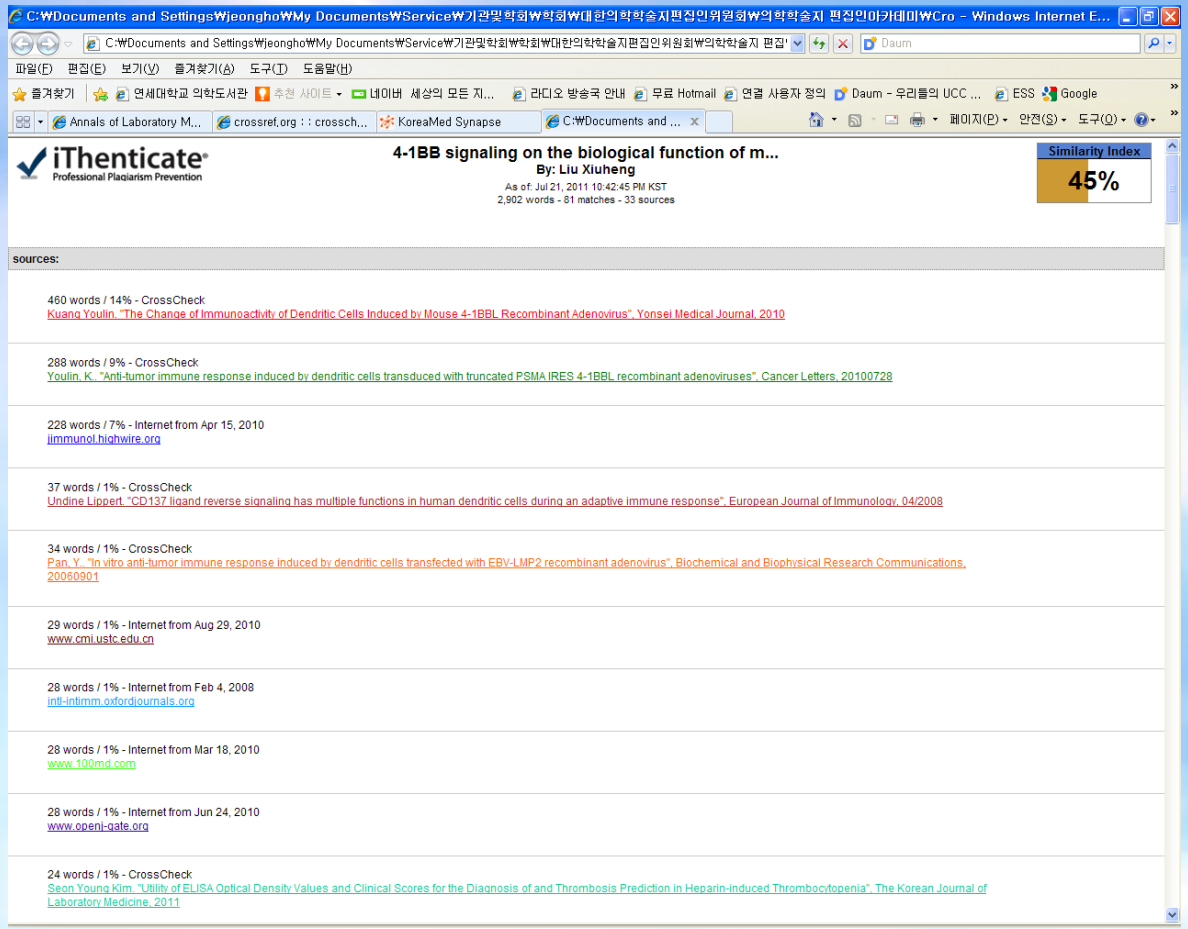

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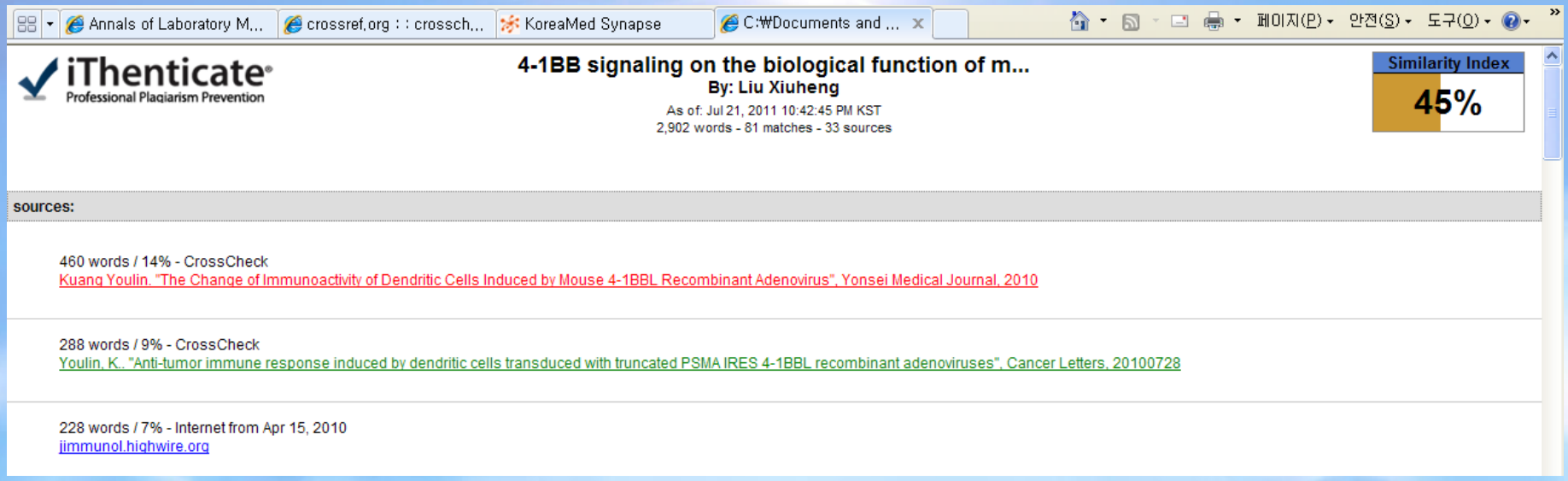

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technology. 2. Isolation and maturation of DCs

Mouse DCs were generated from bone marrow suspensions harvested from 6- 8 week old C57BL/6 mice according to the publication [12] with slight modifications. Briefly, bone-marrow cells were harvested from femurs and tibias, depleted of red blood cells, and washed twice in PBS. Cells were resuspended in a DCs medium consisting of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS) (Gibico, America), 10ng /mL GM-CSF (R&D Systems, Minneapolis, MN, USA), 10ng /mL IL-4 (R&D Systems, Minneapolis, MN,

USA), and 50 mM 4 INFOrang.co., Ltd

2-mercaptoethanol, 100 IU/ml penicillin, and 100 μg/ml streptomycin and cultured (37 ? C, 5% CO2) in 6-well plates at 1×10 6 cells/ 3ml /well. On day 3 and 5 of culture, floating cells were gently removed, and

fresh mGM-CSF/mIL-4-containing medium was added. On day 6, non-adherent cells and loosely adherent proliferating DCs aggregates were collected. Mature DCs were

generated by the inclusion of 10 ng /ml LPS (Sigma) for

another 24h of culture. Then the mature DCs were cultured in 100ug anti-4-1BB Ab, hamster IgG isotypecontrol Ab added or none added medium for another 48 h for following experiments. 3.

Surface marker analysis of DCs For phenotypic analyses by flow cytometry,

4-1BB Ab-triggered DCs (5 × 10 5 )

were stained for 30 min on ice with FITC- or PE-labeled monoclonal antibodies specific for CD11c, CD80, and CD86 (BD Pharmingen, San Diego, CA), after washed three times in phosphate-buffered saline (PBS), the cells were analyzed by flow cytometry. Isotype-matched monoclonal antibodies were used as controls. 4. Cytokine production by DCs For cytokine assays, culture supernatants were harvested and used for enzyme-linked immunosorbent assay (ELISA). A mouse IL-6 Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA) and a mouse IL-12 Quantikine ELISA Kit (R&D Systems) were used to detect IL-6 and IL-12, respectively, following the manufacturer's instructions. 5. Apoptosis analysis

by flow cytometry For apoptosis analysis, 4-1BB Ab-triggered DCs (5 × 10 5 ) were collected and 5 INFOrang.co., Ltd

apoptosis was analyzed by flow cytometry,

staining

with FITC-conjugated annexin V and propidium iodide (PI) according to manufacturer instructions, and

subsequent flow cytometry analysis (Apoptosis Kit, BD Pharmingen, Germany). 6. Western blot analysis DCs were collected

and lysed. The lysates were separated on 10%

SDS-PAGE. After electrophoresis, the protein blots were transferred to a nitrocellulose membrane (Amersham, Waukesha, Wisconsin, USA). The membrane was blocked with 5% non-fat milk in TBST for 1 h and incubated overnight with rabbit anti-

Bcl-2 or rabbit anti-Bcl-xL mAb at 4℃.

After three washes with TBST, the membrane was incubated at 37 ℃ for 1 h with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody diluted with TBST. The detected protein signals were visualized by an enhanced chemilluminescence reaction system. Western blot for β-actin was used as an internal sample.

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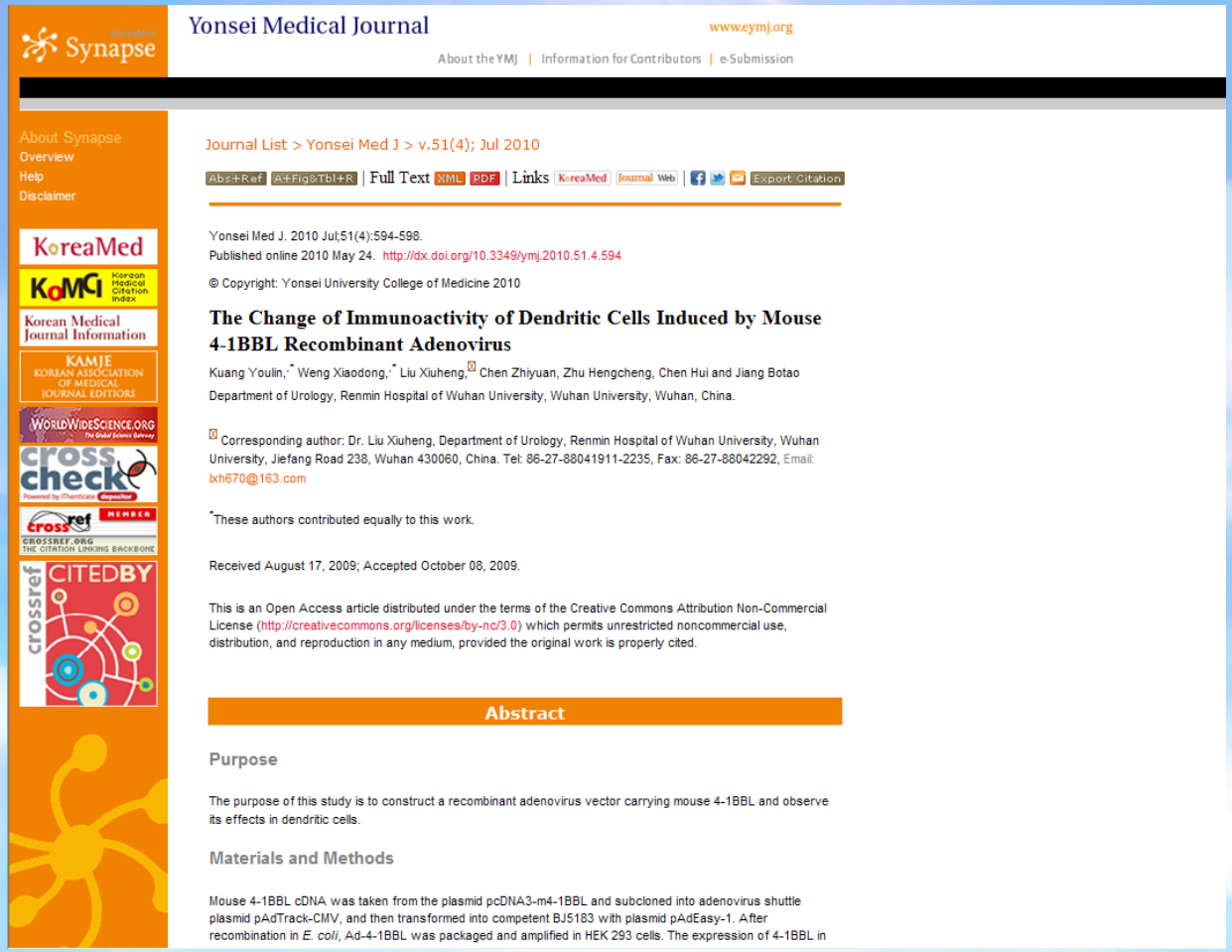

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Published online 2010 May 24. http://dx.doi.org/10.3349/ymj.2010.51.4.594



## The Change of Immunoactivity of Dendritic Cells Induced by Mouse 4-1BBL Recombinant Adenovirus

Kuang Youlin,* Weng Xiaodong,* Liu Xiuheng, Chen Zhiyuan, Zhu Hengcheng, Chen Hui and Jiang Botao
Department of Urology, Renmin Hospital of Wuhan University, Wuhan University, Wuhan, China.

Corresponding author: Dr. Liu Xiuheng, Department of Urology, Renmin Hospital of Wuhan University, Wuhan University, Jiefang Road 238, Wuhan 430060, China. Tel: 86-27-88041911-2235, Fax: 86-27-88042292, Email: bxh670@163.com

*These authors contributed equally to this work.

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**Abstract**

### Purpose

The purpose of this study is to construct a recombinant adenovirus vector carrying mouse 4-1BBL and observe its effects in dendritic cells.

### Materials and Methods

Mouse 4-1BBL cDNA was taken from the plasmid pcDNA3-m4-1BBL and subcloned into adenovirus shuttle plasmid pAdTrack-CMV, and then transformed into competent BJ5183 with plasmid pAdEasy-1. After recombination in *E. coli*, Ad-4-1BBL was packaged and amplified in HEK 293 cells. The expression of 4-1BBL in

# Title Comparison

- **Title submitted to Korean J Lab Med**
  - 4-1BB signaling on the biological function of murine dendritic cells
- **Title published in Yonsei Medical Journal**
  - The change of immunoactivity of dendritic cells induced by mouse 4-1BBL recombinant adenovirus.
    - Yonsei Med J. 2010 Jul;51(4):594-8.

# Author Comparison

- **Authors,** (Submitted to Korean J Lab Med on Jul 21, 2011)
  - Kuang Youlin, Zhang Jianwei, Weng Xiaodong, Liu Xiuheng, Zhu Hengchen, Chen Zhiyuan
    - Department of Urology, Renmin Hospital of Wuhan University, Wuhan, China
- **Authors, (**Yonsei Med J. 2010 Jul;51(4):594-8)
  - Kuang Youlin,* Weng Xiaodong,* Liu Xiuheng, Chen Zhiyuan, Zhu Hengcheng, Chen Hui, and Jiang Botao
    - Department of Urology, Renmin Hospital of Wuhan University, Wuhan University, Wuhan, China.

# Abstract Background Comparison

- **제출한 논문** (Submitted to Korean J Lab Med on Jul 21, 2011)
  - **Background:** 4-1BB signal has profound effects on T cell induced cell immune response, but its biological function on dendritic cells (DCs) has remained largely uncharacterized. Here, we investigated the function of 4-1BB on murine DCs with an agonistic mAb to 4-1BB.
- **출판된 유사 논문 (**Yonsei Med J. 2010 Jul;51(4):594-8)
  - **Purpose:** The purpose of this study is to construct a recombinant adenovirus vector carrying mouse 4-1BBL and observe its effects in dendritic cells.

# Abstract Methods Comparison

- **제출한 논문** (Submitted to Korean J Lab Med on Jul 21, 2011)
  - **Methords:** IL-6 and IL-12 production were assessed by enzyme-linked immunosorbent assay (ELISA) and co-stimulatary moleculs (CD80 and CD86) on DCs were analyzed by flow cytometry. Bcl-2 and Bcl-xL expression were detected by western blot.
- **출판된 유사 논문** (Yonsei Med J. 2010 Jul;51(4):594-8)
  - **Materials and Methods:** Mouse 4-1BBL cDNA was taken from the plasmid pcDNA3-m4-1BBL and subcloned into adenovirus shuttle plasmid pAdTrack-CMV, and then transformed into competent BJ5183 with plasmid pAdEasy-1. After recombination in E. coli, Ad-4-1BBL was packaged and amplified in HEK 293 cells. The expression of 4-1BBL in Ad-4-1BBL-transfected mouse prostate cancer cell line RM-1 was detected by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. After the co-culture of dendritic cells (DCs) with Ad-4-1BBLtransfected RM-1 cells, interleukin (IL)-6 and IL-12 production were assessed by enzyme-linked immunosorbent assay (ELISA) and co-stimulatary moleculs (CD80 and CD86) on DCs were analyzed by flow cytometry.

# Abstract Results Comparison

- **제출한 논문** (Submitted to Korean J Lab Med on Jul 21, 2011)
  - **Results:** We found that 4-1BB was strongly expressed on DCs during the maturation, and triggering 4-1BB increased the secretion of IL-6 and IL-12 and up-regulation of co-stimulatory molecules (CD80, CD86) from DCs, indicating agonistic mAb to 4-1BB can directly improve the activation of DCs. Moreover, triggering 4-1BB induced higher survival rate of DCs compared to that of hamster IgG isotypecontrol, which was owing to the up-regulated expression of Bcl-2 and Bcl-xL. To further assess the role of 4-1BB on DCs stimulating T cell proliferation, allogeneic mixed lymphocyte reactions was analyzed. The agonistic anti-4-1BB mAb induced higher T cell proliferation
- **출판된 유사 논문** (Yonsei Med J. 2010 Jul;51(4):594-8)
  - **Results:** The levels of IL-6 (3,960 pg/mL) and IL-12 (249 pg/mL) production in Ad-m4-1BBL-pulsed DCs were more than those in none-pulsed DCs. The differences were statistically significant ($p < 0.05$). The expression of co-stimulatary molecules (CD80 and CD86) was up-regulated in Ad-m4-1BBL-pulsed DCs.

# Abstract Conclusions Comparison

- **제출한 논문** (Submitted to Korean J Lab Med on Jul 21, 2011)
  - **Conclussions:** These results suggest that 4-1BB on DCs could effect the duration, DC-T interaction, and immunogenicity.
- **출판된 유사 논문** (Yonsei Med J. 2010 Jul;51(4):594-8)
  - **Conclusion:** The results indicated the recombinant mouse 4-1BBL can effectively activate DCs.

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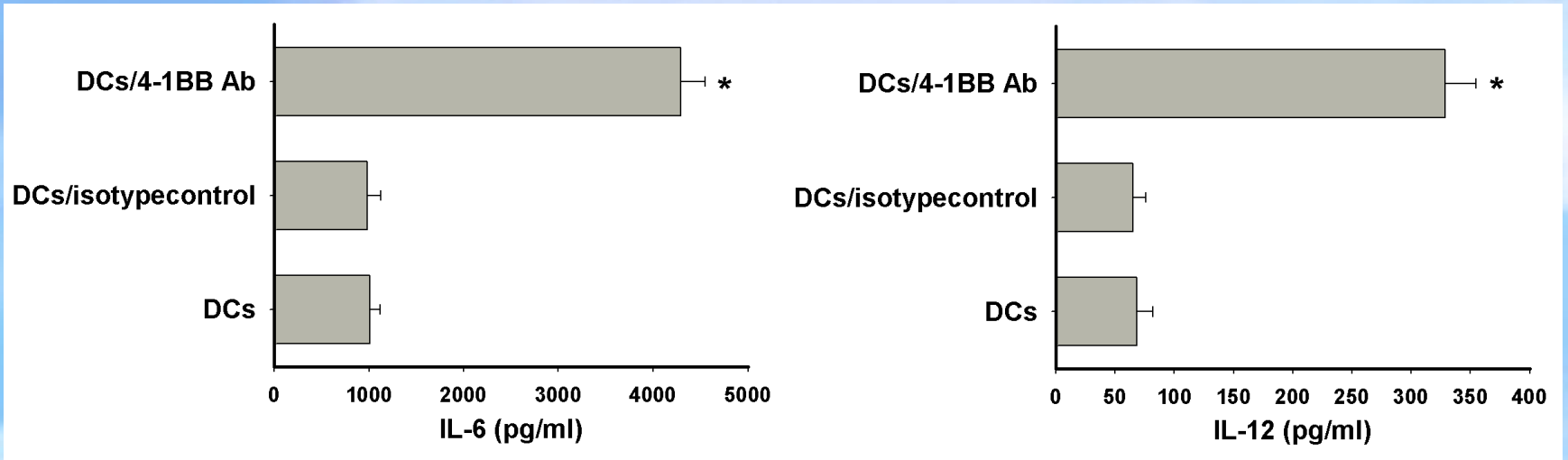

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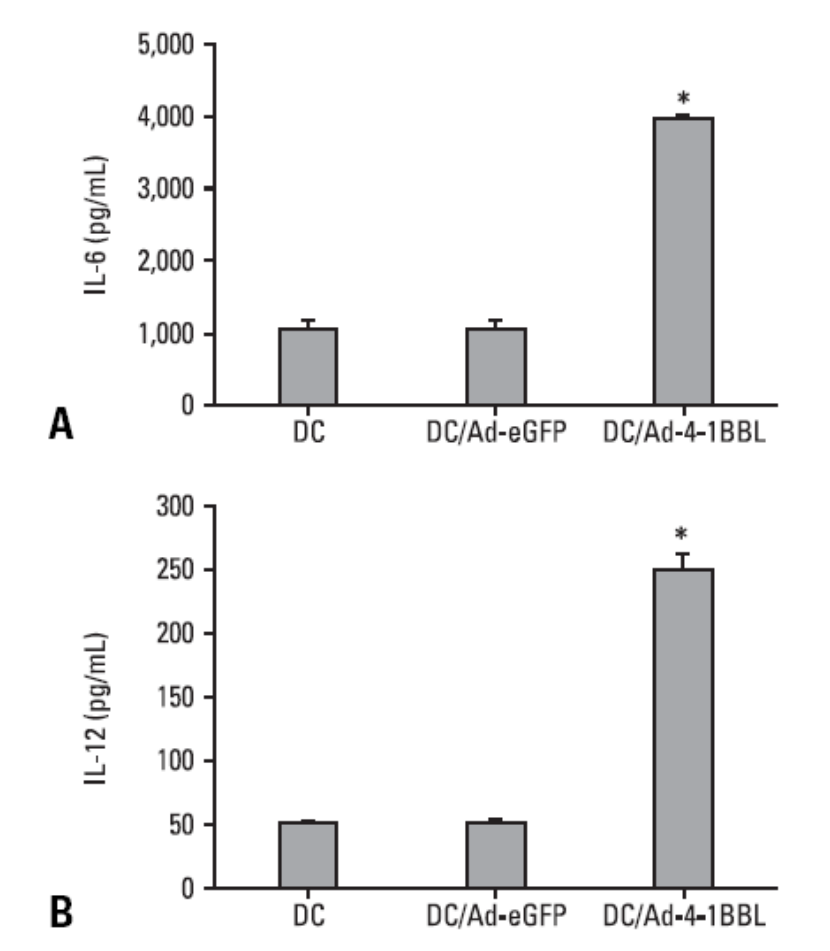


- Youlin K, Xiaodong W, Xiuheng L, Zhiyuan C, Hengcheng Z, Hui C, Botao J. The change of immunoactivity of dendritic cells induced by mouse 4-1BBL recombinant adenovirus.

# 중복출판의 유형

- 위 예는 유사논문인 Yonsei Med J. 2010 Jul;51(4):594-8 의 인용 없이 유사점이 많아서 다음과 같은 복합 유형의 중복출판으로 간주하였다.
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## The change of immunoactivity of dendritic cells induced by mouse 4-1BBL recombinant adenovirus.

Youlin K, Xiaodong W, Xiuheng L, Zhiyuan C, Hengcheng Z, Hui C, Botao J.

Department of Urology, Renmin Hospital of Wuhan University, Wuhan University, Wuhan 430060, China.

### Abstract

**PURPOSE:** The purpose of this study is to construct a recombinant adenovirus vector carrying mouse 4-1BBL and observe its effects in dendritic cells.

**MATERIALS AND METHODS:** Mouse 4-1BBL cDNA was taken from the plasmid pcDNA3-m4- 1BBL and subcloned into adenovirus shuttle plasmid pAdTrack-CMV, and then transformed into competent BJ5183 with plasmid pAdEasy-1. After recombination in E.coli, Ad-4-1BBL was packaged and amplified in HEK 293 cells. The expression of 4-1BBL in Ad-4-1BBL-transfected mouse prostate cancer cell line RM-1 was detected by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. After the co-culture of dendritic cells (DCs) with Ad-4-1BBL-transfected RM-1 cells, interleukin (IL)-6 and IL-12 production were assessed by enzyme-linked immunosorbent assay (ELISA) and co-stimulatary molecules (CD80 and CD86) on DCs were analyzed by flow cytometry.

**RESULTS:** The levels of IL-6 (3,960 pg/mL) and IL-12 (249 pg/mL) production in Ad-m4-1BBL-pulsed DCs were more than those in none-pulsed DCs. The differences were statistically significant ($p < 0.05$). The expression of co-stimulatary molecules (CD80 and CD86) was up-regulated in Ad-m4-1BBL-pulsed DCs.

**CONCLUSION:** The results indicated the recombinant mouse 4-1BBL can effectively activate DCs.

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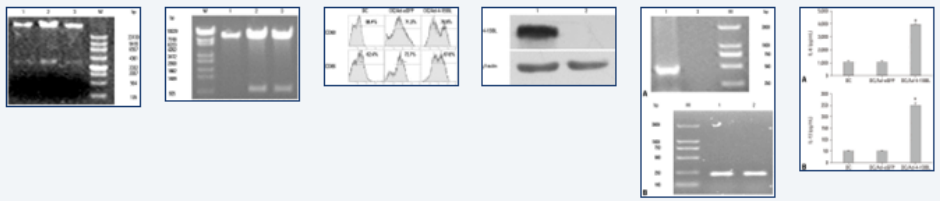

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Anti-tumor immune response induced by dendritic cells transduced with [Cancer Lett. 2010]

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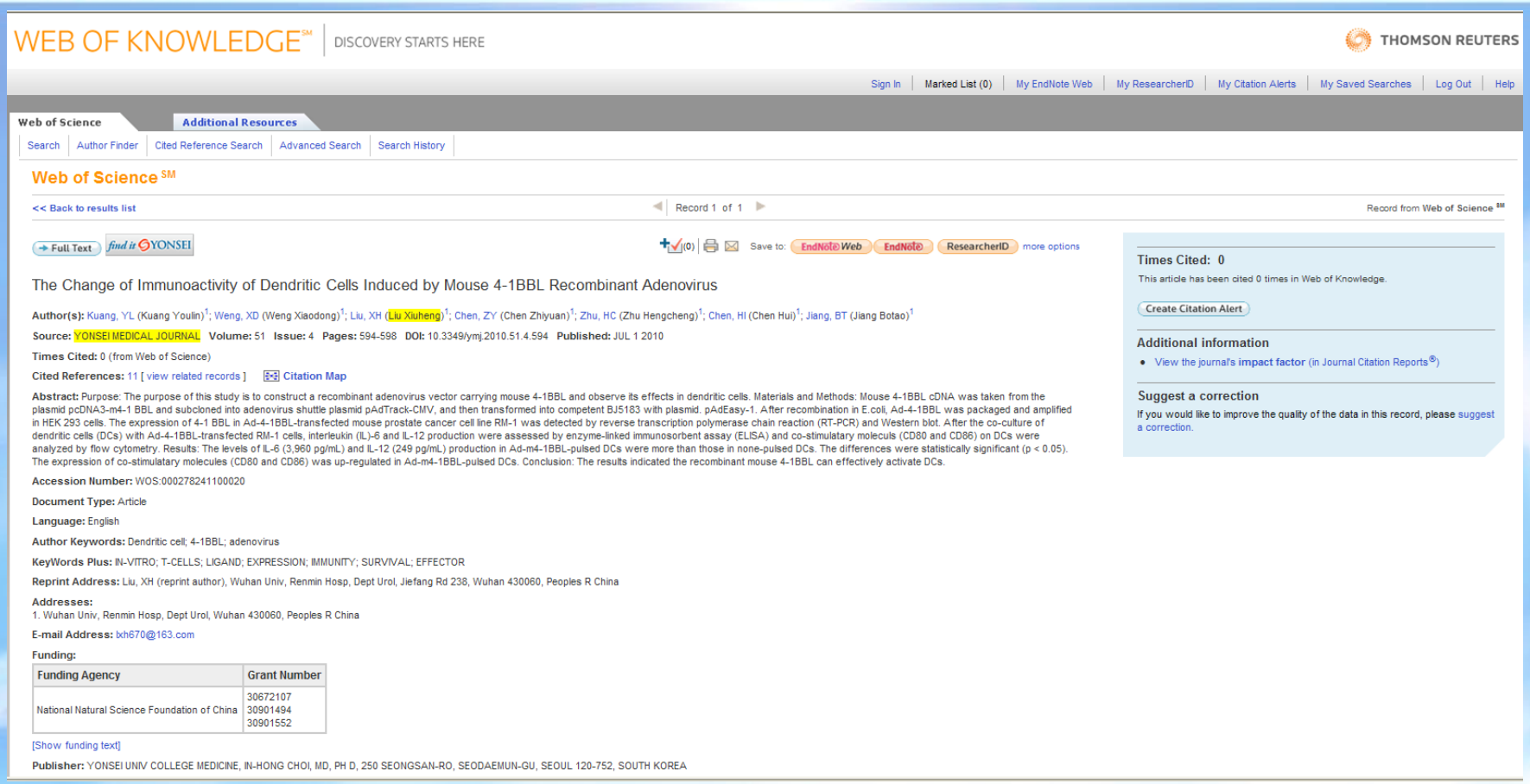

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The Change of Immunoactivity of Dendritic Cells Induced by Mouse 4-1BBL Recombinant Adenovirus

**Author(s):** Kuang, YL (Kuang Youlin)[1]; Weng, XD (Weng Xiaodong)[1]; Liu, XH (Liu Xiuheng)[1]; Chen, ZY (Chen Zhiyuan)[1]; Zhu, HC (Zhu Hengcheng)[1]; Chen, HI (Chen Hui)[1]; Jiang, BT (Jiang Botao)[1]

**Source:** YONSEI MEDICAL JOURNAL **Volume:** 51 **Issue:** 4 **Pages:** 594-598 **DOI:** 10.3349/ymj.2010.51.4.594 **Published:** JUL 1 2010

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**Abstract:** Purpose: The purpose of this study is to construct a recombinant adenovirus vector carrying mouse 4-1BBL and observe its effects in dendritic cells. Materials and Methods: Mouse 4-1BBL cDNA was taken from the plasmid pcDNA3-m4-1 BBL and subcloned into adenovirus shuttle plasmid pAdTrack-CMV, and then transformed into competent BJ5183 with plasmid. pAdEasy-1. After recombination in E.coli, Ad-4-1BBL was packaged and amplified in HEK 293 cells. The expression of 4-1 BBL in Ad-4-1BBL-transfected mouse prostate cancer cell line RM-1 was detected by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. After the co-culture of dendritic cells (DCs) with Ad-4-1BBL-transfected RM-1 cells, interleukin (IL)-6 and IL-12 production were assessed by enzyme-linked immunosorbent assay (ELISA) and co-stimulatary molecuis (CD80 and CD86) on DCs were analyzed by flow cytometry. Results: The levels of IL-6 (3,960 pg/mL) and IL-12 (249 pg/mL) production in Ad-m4-1BBL-pulsed DCs were more than those in none-pulsed DCs. The differences were statistically significant ($p < 0.05$). The expression of co-stimulatary molecules (CD80 and CD86) was up-regulated in Ad-m4-1BBL-pulsed DCs. Conclusion: The results indicated the recombinant mouse 4-1BBL can effectively activate DCs.

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**Document Type:** Article

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**Reprint Address:** Liu, XH (reprint author), Wuhan Univ, Renmin Hosp, Dept Urol, Jiefang Rd 238, Wuhan 430060, Peoples R China

**Addresses:**
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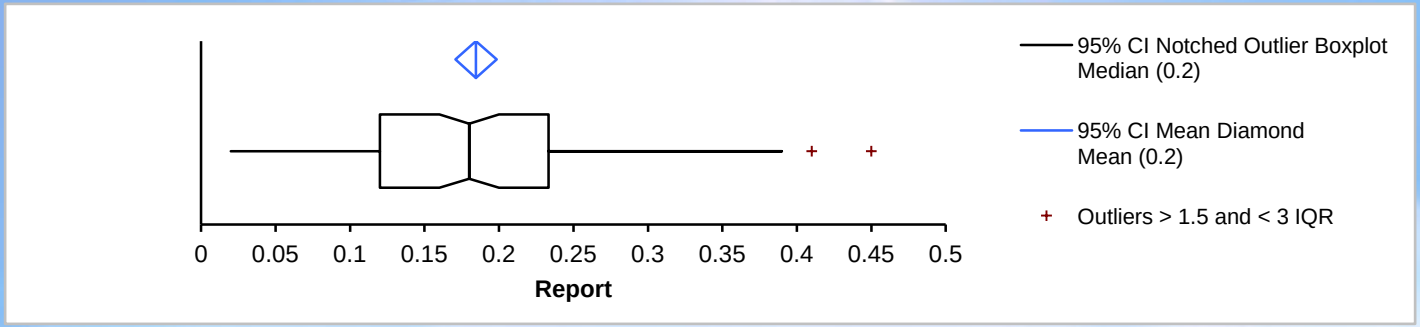
강남세브란스병원 GANGNAM SEVERANCE HOSPITAL

# iThenticate Report (Jul '11 to Jan '12)




| | | |
|---|---|---|
| n | 141 | |
| Mean | 18.5% | |
| Median | 18.0% | |
| Percentile 0th | 2.0% | (minimum) |
| 25th | 12.0% | (1st quartile) |
| 50th | 18.0% | (median) |
| 75th | 23.3% | (3rd quartile) |
| 100th | 45.0% | (maximum) |

# Editorial Turnaround Time (Median Days)

| 항목 | 2011년 1월~3월 | 2011년 4월~6월 | 2011년 7월~9월 | 2011년 10월~12월 |
|---|---|---|---|---|
| 1. 모든 심사위원에게 심사의뢰 완료일 | 8 일 | 8 일 | 12.5 일 | 7 일 |
| 2. 게재허가 추천완료일 | 91 일 | 94 일 | 67 일 | 38.5 일 |
| 3. 게재허가 완료일 | 102 일 | 136.5 일 | 117 일 | 50.5 일 |
| 4. 게재불가 완료일 | 25 일 | 28 일 | 23 일 | 17 일 |
| 5. 출판교정 완료일 | 156 일 | 156.5 일 | 130.5 일 | 11 일 |
| 6. 출판일 완료일 | 231 일 | 244 일 | 222.5 일 | 97.5 일 |

# Summary

- CrossCheck (iThenticate) was useful to check plagiarism and save the loss of editorial work
- We need careful review of the manuscript which exceeds the threshold of similarity report.
- Although CrossCheck (iThenticate) is not complete, its usage may prevent many cases of future plagiarism.

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