

# THE EFFECT OF CONDITIONED MEDIUM ADIPOSE DERIVED MESENCHYMAL STEM CELLS IN UROTHELIAL CARCINOMA CULTURE CELLS VIABILITY

<sup>1</sup>Suryo Prasetyo Tribowo, <sup>1</sup>Furqan Hidayatullah, <sup>2</sup>Indra Bachtiar, <sup>3</sup>Arif Rachman, <sup>4</sup>Indri Lakhsmi Putri, <sup>5</sup>Fabio Castiglione, <sup>1</sup>Doddy M. Soebadi, <sup>1,6</sup>Lukman Hakim.

<sup>1</sup>Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya.

<sup>2</sup>ReGeniC Laboratory, PT Biofarma Adiluhung and Stem cell & Cancer Institute, PT Kalbe Farma Tbk, Jakarta.

<sup>3</sup>Indonesian Naval Dentistry Institute, Surabaya.

<sup>4</sup>Department of Plastic Reconstructive and Esthetic, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital, Surabaya.

<sup>5</sup>Urological research institute, Department of Urology, University Vita-Salute San Raffaele, Milan, Italy.

<sup>6</sup>Center for Stem Cell research and development/Universitas Airlangga, Surabaya.

## ABSTRACT

**Objective:** This study aimed to give a perspective in CM-ADMSCs effect in urothelial bladder cancer viability. **Material & Methods:** Human bladder cell carcinoma type 5637 was used as the subject of this in vitro study. This study contains four different groups: untreated control group, Culture medium: hADMSCs with 1:1, 1:2, and 1:4 concentration group. Each group consists of 6 replications to prevent bias of the study. Viability was determined with MTT assay methods and evaluation performed after 48 h exposure of conditioned medium. **Results:** A post hoc test was conducted to analyze the data. The 5637 bladder cancer cell line demonstrated significantly decreased viability after exposure to culture medium: CM-hADMSCs 1:1 ( $p: 0.002$ ) compared to the negative control group, but there are no significant differences in viability between the control groups with groups that were exposed to culture medium: CM-hADMSCs 1:2 and culture medium: CM-hADMSCs 1:4 with  $p: 0.480$  and  $p: 0.060$  respectively. **Conclusion:** Decreased viability of urothelial bladder cancer cells after exposure to CM-hADMSCs occurs at a concentration of 1:1 and Dosage addition more than 1:1 concentration doesn't give any advantages.

**Keywords:** Bladder cancer, conditioned medium, adipose-derived mesenchymal stem cells, viability, MTT.

## ABSTRAK

**Tujuan:** Penelitian ini diharapkan dapat memberikan perspektif dalam mengetahui peran paparan conditioned medium human Adipose Derived Mesenchymal Stem Cells (CM-hADMSCs) terhadap viabilitas sel kanker buli urothelial. **Bahan & Cara:** Penelitian ini menggunakan sel kultur karsinoma urothelial manusia tipe 5637 yang dibiakkan secara in vitro. Pada penelitian ini, kelompok penelitian dibagi menjadi 4 dengan 6 replikasi pada tiap kelompok, yaitu kelompok kontrol tanpa perlakuan, kelompok perlakuan dengan pemberian medium kultur : CM-hADMSCs 1:1, 1:2, dan 1:4. Metode MTT assay digunakan untuk mengetahui viabilitas sel karsinoma buli. Waktu pengamatan viabilitas dilakukan setelah paparan dengan conditioned medium selama 48 jam. **Hasil:** Analisis post hoc antara semua grup digunakan untuk menunjukkan hasil yang perbedaan yang signifikan antara kelompok. Sel Kultur Karsinoma Urothelial Buli 5637 menunjukkan penurunan viabilitas yang signifikan setelah pemberian medium kultur : CM-ADMSCs 1:1 dengan kelompok kontrol ( $p: 0.002$ ), namun tidak didapatkan perbedaan signifikan antara pemberian medium kultur : CM-ADMSCs 1:2 dan 1:4 dengan kelompok kontrol ( $p: 0.480$  dan  $p: 0.060$ ). **Simpulan:** Penurunan viabilitas sel kanker buli urothelial setelah terpapar medium kultur sel-CM-hADMSCs terjadi pada konsentrasi 1:1 dan pemberian dosis tambahan tidak memberikan keuntungan.

**Kata Kunci:** Kanker buli, conditioned medium, adipose derived mesenchymal stem cells, viabilitas, MTT.

Correspondence: Lukman Hakim; c/o: Department of Urology, Faculty of Medicine/Universitas Airlangga, Soetomo General Hospital Surabaya. Jl. Mayjen. Prof. Dr. Moestopo 6-8 Surabaya 60286. Phone: +62 31 5501318; Fax: +62 31 5024971. Mobile phone: +6281221447576. Email: lukman0908@gmail.com.

## INTRODUCTION

Bladder cancer is one of the most common urinary tract cancer with increased incidence.<sup>1</sup> Bladder cancer is the 12<sup>th</sup> most common cancer with a 15% incidence rate per year in Indonesia.<sup>2</sup> Currently, bladder cancer management using intravesical instillation, radical cystectomy, radiotherapy, and chemotherapy, depending on the type and stage of bladder cancer.

Mesenchymal stem cells (MSCs) derived from adipose tissue, bone marrow, cord blood, and other tissues, have recently attracted much attention as potential therapeutic agents in various diseases because of their trans-differentiation capacity.<sup>3</sup> MSCs can migrate specifically to tumors and able to be used as a tumor-tropic vector with anti-cancer activity.<sup>4-5</sup> The use of the conditioned medium (CM) from MSC-sourced secretome stem-cell in regenerative medicine provides key advantages. CM is more economical than MSC since mass-production is possible and avoids excessive cell collection procedures.<sup>6</sup>

Recently there was very little research on the effect of CM-ADMSC on bladder cancer cells, especially on urothelial cancer cells. In this study, we want to investigate the effect of CM-hADMSCs, to measure viability and proliferation rates in urothelial bladder cancer cells exposed to human adipose mesenchymal stem cells.

## OBJECTIVE

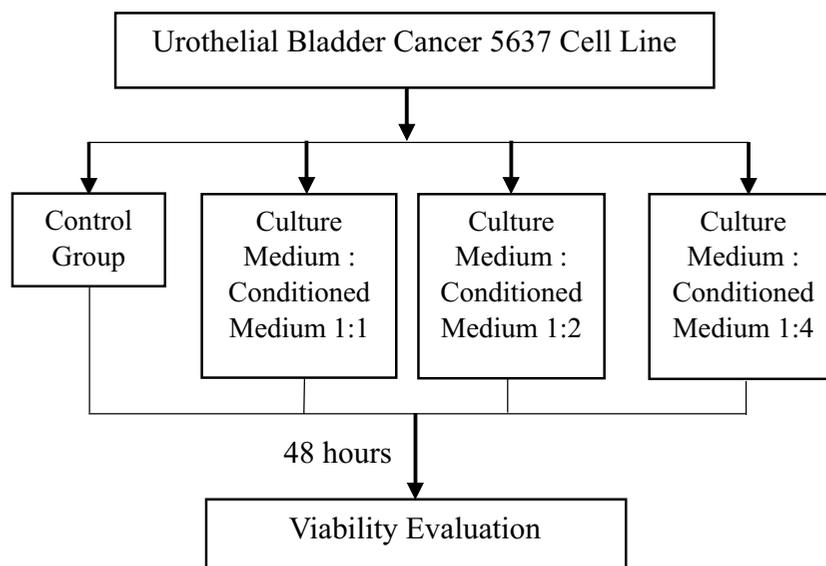
This study aimed to investigate the effect of conditioned medium Adipose-Derived Mesenchymal Stem Cells (CM-ADMSCs) exposure on viability in urothelial bladder cancer cells.

## MATERIAL & METHODS

This in vitro experimental study is using human bladder cell carcinoma type 5637 cell line. In this study, the conditioned medium of MSCs was derived from adipose tissues. The study group was divided into 4 groups: untreated control group, Culture medium: hADMSCs with 1:1 concentration group, Culture medium: hADMSCs with 1:2 concentration group, and the Culture medium: hADMSCs with 1:4 concentration group.

**Isolation and identification of MSCs.** MSCs are isolated from women who underwent a caesarean section and fat biopsy is performed intraoperatively. The harvesting process, characterization, and differentiation of MSC in this research are according to (Rahman et al., 2018) without any further modification.<sup>7</sup>

**Cells Culture.** 5637 cell line are low-grade primary bladder cancer. Cell lines Bladder Cancer 5637 are purchased from CLS® (Cell Line Service) GmbH (Eppelheim, Germany). 5637 cells were cultured in RPMI 1640 medium. ADMSCs were



**Figure 1.** Research conceptual framework.

cultured in alpha-MEM supplemented with 10% FBS 10 ng/mL Cells were stored in the incubator and maintained at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

**Conditioned Medium Harvesting.** ADMSCs were cultured as previously described. When the cells grew to 80–90% confluence at 3rd passage, the media from ADMSCs were harvested as conditioned media and stored at –80°C until use.

**MTT Analysis.** 5637 cells cultured in RPMI 1640 medium as the negative control. The culture medium was replaced every 24 h. The 5637 cells then divided into three groups and cultured with proportions of the culture medium and conditioned media were 1:1, 1:2, and 1:4 for 48 h and MTT (Bio Basic, USA) analysis was generated on M96 plate to measure the 5637 cells viability. One extra well with six sample replicant were used in this study to measure the OD of RPMI medium culture to prevent bias in the OD measurement. MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added until reaches final concentration of 0.5 mg/mL for each test well and was maintained for 4 h. The supernatant was separated, and 150 µl of dimethyl sulfoxide (Sigma) was added and stirred at room temperature for 5 min. The microplate was read at an absorbance value of 570 nm with a universal microplate spectrophotometer (Sunrise Australia). As control medium is wells without cells considered as the blank control, and each group had 6 replicate wells. The viability of 5637 cells was

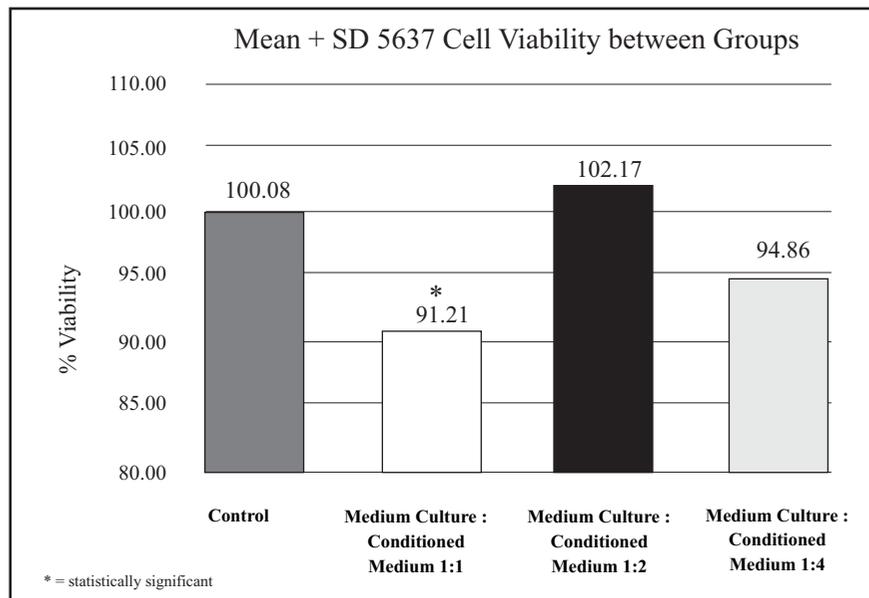
formulated as follows:  $\text{viability (\%)} = \frac{\text{OD570 value of the experiment's group} - \text{OD570 value of medium RPMI only}}{\text{OD570 value of control group} - \text{medium RPMI only}} \times 100$ .

**Statistical Analysis.** The results from the research were collected in data collection sheets, then grouped and presented in the tables and diagrams. Data analysis was performed using the SPSS 17 program. One Way ANOVA was used for comparative analysis of apoptosis between the group with a significant value of  $p < 0.05$ . The analysis was continued with a post hoc LSD test, to determine the differences in each group.

**RESULTS**

The data distribution on the average viability of bladder cancer cells is normally distributed with  $p > 0.05$ . One-Way ANOVA parametric analysis test results showed a significant difference between treatment groups. With  $p < 0.05$ . The results show that there is a significant difference in minimum viability in one study group.

One-Way ANOVA parametric analysis test revealed a significant difference between treatment groups continued with the post hoc test to compare the specific differences of the mean between the study groups. From the results of the post hoc LSD test, there was no difference in viability between the control group with groups that were exposed to culture medium: CM-hADMSCs 1:2 and culture



**Figure 2.** Mean ± SD of Apoptosis Index.

**Table 1.** LSD post-hoc of Bladder Cancer 5637 cell line viability between groups after 48 hours.

Groups	Mean Difference	95% CI		p
		Lower Bound	Upper Bound	
Control vs 1:1 CM-hADMSCs	8.86667	0.0129	0.0718	0.002*
Control vs 1:2 CM-hADMSCs	2.09500	-0.334	0.0254	0.418
Control vs 1:4 CM_hADMSCs	5.04333	_0.081	0.040	0.060
1:1 CM-hADMSCs vs 1:2 CM-hADMSCs	10.96167	-0.075	-0.016	0.000*
1:1 CM-hADMSCs vs 1:4 CM-hADMSCs	3.82333	-0.060	-0.016	0.147
1:2 CM-hADMSCs vs 1:4 CM-hADMSCs	7.13833	-0.014	0.0447	0.011*

\* p < 0,05: statistically significant.

medium: CM-hADMSCs 1:4 (p>0.05). Mean viability analysis revealed that there were significant differences in the viability between the culture medium treatment groups: CM-HADMSCs 1:1 to the control group and concentration of 1:2 studies with p<0.05. There was no difference in mean viability at the dose of culture medium: CM-hADMSCs 1:2 compared to the treatment groups exposed to culture medium: CM-hADMSCs 1:1 and 1:4 (Table 1).

## DISCUSSION

MSCs have potential therapeutic effects and multipotent agents used as regenerative therapies and degenerative disorders. MSCs have secretions/metabolites consist of cytokines and growth factors that can create conditions to trigger paracrine signals or direct effects on cells. This condition provides the potential use of MSCs for the treatment of urothelial cancer.

According to Malgorzata et al, their study inconsistently revealed that MSC can reduce the viability of T24 bladder cancer cell line through an inhibitory effect on the cell cycle that triggers the arrests of the cell cycle in G2 phase. Conversely, exposure to CM-hADMSCs can induce an increase in viability of bladder cancer cell line 5637 and T-1376.<sup>8</sup> In this study CM hADMSCs can reduce the viability of bladder cancer cell line 5637. Culture Media: CM ADMSCs 1:1 can reduce viability by 8.5% and statistically significant (p= 0.002), while in doses of 1:2 and 1:4, there is no difference in the viability of 5637 bladder cancer cells compared to the control group (p= 0.418 and p= 0.06 respectively).

These paradoxical results in the administration of MSC indicate that the interaction mechanism and response between MSC and bladder cell is still unclear. Inconsistent findings in this study can be influenced by various factors. The difference in sensitivity to conditioned medium exposure can be caused by the profile expression of the genes contained which consists of immunological, functional, and biochemical components.<sup>8</sup>

Otsu et al found that an MSC ratio of 1:1 or 1:3 can cause cytotoxicity but injection together with 10% MSC does not cause cytotoxicity.<sup>9</sup> However, the results in this study differ from the study conducted by Hou et al who showed the addition of conditioned medium MSCs doses can inhibit the proliferation of human Hepatoma HepG2 cells.<sup>10</sup> In that study, Hou et al. found that culture together with CM-hADMSCs and HepG2 cells with concentrations of 40%, 60%, 80%, 100% can reduce cell viability but at a concentration of 20%, no significant difference was found. This revealed that the administration of MSCs can be dose-dependent.

Decreased viability can be determined by several factors, one of which is a decrease in the rate of cell proliferation. MSCs obtained from bone marrow revealed antiproliferative effects on hematopoietic and non-hematopoietic cell tumors.<sup>11</sup> Yang et al. found MSCs and CM-MSCs can inhibit glioma cell proliferation through increased cell cycle inhibitors protein p21, p27, and p53. They also found that MSCs and CM-MSCs could induce cell apoptosis mediated by upregulation of Caspases 3 and 9 and downregulation of XIAP and Survivin.<sup>12</sup> MSC also secreted factors such as VEGF which provided blood vessel development. MSC can migrate and produce reactive oxygen species and cause apoptosis from endothelial cells.<sup>9</sup>

The mechanism related to the interaction of cell cancer, especially bladder cancer and CM hADMSCs does require further research. Understanding of the content present in the metabolites of stem cells as well as the behavior of cancer cells and niche tumors can provide promising developments for alternative therapies for cancer cells, especially cancer cells in the urinary tract.

## LIMITATION

This research is in vitro experimental study. The dosage and duration given in this study are limited. Further research that focuses on the concentration of CM-hADMSCs dose and duration of treatment was needed to reduce research sample bias.

## CONCLUSION

CM-hADMSCs suppress urothelial bladder cancer viability in a dosage-dependent manner. Decreased viability of urothelial bladder cancer cells after exposure to cell growth media and CM-hADMSCs occurs at concentrations of 1:1. Dosage addition more than 1:1 concentration doesn't give any advantages.

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