

# Isolasi, kultur dan karakterisasi giant cell tumor dari jaringan pasien primer: uji sitotoksitas dan mekanisme cedera akibat beragam zat kimia = Isolation, culture and characterization of giant cell tumor from primary human patients: cytotoxicity test and mechanism of injury to various chemical agents / Akbar Rizki Beni Asdi

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## Abstrak

### <b>ABSTRAK</b><br>

Latar Belakang Giant cell tumor (GCT) merupakan tumor jinak yang bersifat lokal agresif destruktif. Tumor ini memiliki rekurensi yang tinggi sebanyak 25-50% setelah tindakan pembedahan. Berbagai macam pemberian zat kimia lokal sebagai terapi ajuvan, telah digunakan pada tatalaksana pembedahan. Namun perbandingan efektifitas untuk masing-masing zat kimia ini belum diketahui. Studi mengenai sitotoksitas dan mekanisme kematian sel dengan membandingkan pemberian etanol dan H<sub>2</sub>O<sub>2</sub> pada sel GCT tulang secara in vitro masih sedikit dan belum ada di Indonesia.

Metode Penelitian ini merupakan studi in vitro eksperimental dengan mengambil empat sampel jaringan tumor dari pasien yang didiagnosis GCT tulang dan dilakukan isolasi-kultur sel. Cell line yang didapatkan dikarakterisasi melalui analisis morfologi serta pemeriksaan ekspresi penanda gen Nanog dan Oct 4 dengan Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Sel yang telah 80% konfluens dilakukan terapi dengan H<sub>2</sub>O<sub>2</sub> 1%, 3%, 5% dan etanol 75%, 85%, 95% selama 10 menit serta dosis in vitro H<sub>2</sub>O<sub>2</sub> (0,003%, 0,005%, 0,01%, 0,03%, 0,1%, 0,3%) selama 5 menit serta inkubasi selama 24 jam. Morfologi sel dievaluasi dibawah mikroskop cahaya dengan membandingkan kontrol dan setelah pemberian zat kimia, viabilitas sel dihitung menggunakan automatic cell counter serta toksisitas sel dinilai dengan uji Annexin V dan Propidium Iodida (PI) pada flow cytometry.

Hasil Kultur jaringan sel GCT dengan metode eksplan dan kolagenase mempunyai angka keberhasilan yang sama dalam mendapatkan cell line GCT. Namun metode eksplan membutuhkan waktu yang lebih cepat dan memiliki jumlah sel yang lebih banyak. Sel yang tumbuh dari jaringan GCT terkarakterisasi dengan analisis morfologi serta ekspresi gen Oct 4 dan Nanog. Viabilitas sel GCT menurun secara signifikan setelah paparan terhadap dosis klinis H<sub>2</sub>O<sub>2</sub> 1% (p = 0,046), H<sub>2</sub>O<sub>2</sub> 3% (p = 0,043), dan H<sub>2</sub>O<sub>2</sub> 5% (p = 0,043) selama 10 menit dibandingkan dengan kontrol. Tidak ada perbedaan yang bermakna untuk viabilitas sel antara konsentrasi H<sub>2</sub>O<sub>2</sub> 1%, 3% dan 5%. Sementara pada konsentrasi in vitro (0,003%, 0,005%, 0,01%, 0,03%, 0,1%, 0,3%), konsentrasi H<sub>2</sub>O<sub>2</sub> 0,3% (p < 0,001) selama 5 menit memiliki efektivitas paling baik dalam sterilisasi GCT secara in vitro. Terdapat fenomena fiksasi sel setelah pemberian etanol pada semua konsentrasi. Dari uji RT-PCR didapatkan penurunan ekspresi gen Oct 4 dan Nanog seiring dengan peningkatan konsentrasi H<sub>2</sub>O<sub>2</sub> pada dosis in vitro. Flow cytometry dengan marker Annexin V dan propidium iodide (PI) didominasi oleh marker PI yang menunjukkan kematian sel akibat nekrosis dengan persentase terbesar pada konsentrasi 0,3%.

Kesimpulan Eksplan merupakan metode terbaik dalam isolasi dan kultur sel GCT. Semua sel hasil isolasi dan kultur terkarakterisasi sebagai GCT. Pemberian ajuvan kimia lokal dengan dosis klinis H<sub>2</sub>O<sub>2</sub> konsentrasi 1%, 3%, dan 5% selama 10 menit secara in vitro mempunyai efektivitas yang sama dalam

membunuh sel GCT. Sedangkan konsentrasi H<sub>2</sub>O<sub>2</sub> 0,3% selama 5 menit merupakan terapi optimal dalam sterilisasi GCT secara in vitro dengan mekanisme kematian nekrosis sel.

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<b>ABSTRACT</b><br>

**Background** Giant cell tumor (GCT) is a benign, aggressive local tumor with high tendency to recur after surgery. Various chemicals have been used as an adjuvant treatment for GCT. However, the comparative effect of these chemicals remains unclear. To date, there are no studies about the cytotoxicity and mechanism of injury to etanol and H<sub>2</sub>O<sub>2</sub> in GCT in Indonesia especially in vitro experiment. The present study aims to find the best method to isolation and culture of GCT from primary human patients, the optimal treatment of etanol and H<sub>2</sub>O<sub>2</sub> for reducing GCT recurrence.

**Methods** This is an experimental in vitro study that took four tumor tissue samples from patients diagnosed with bone GCT and conducted cell-culture isolation. Cell line characterized by morphology, gene markers Nanog and Oct 4 expression with Polymerase Chain Reaction (RT-PCR) Reverse Transcriptase was obtained. Cells that had 80% confluence were treated with H<sub>2</sub>O<sub>2</sub> 1%, 3%, 5% and etanol 75%, 85%, 95% for 10 minutes and in vitro doses of H<sub>2</sub>O<sub>2</sub> (0.003%, 0.005%, 0.01%, 0.03 %, 0.1%, 0.3%) for 5 minutes and were incubated for 24 hours. Cell morphology was evaluated under a light microscope by comparing the morphology of controls and after exposure a chemical agents, cell viability was calculated using automatic cell counter and cell toxicity was assessed by Annexin V and Propidium Iodida (PI) on flow cytometry.

**Results** Collagenase and explant methods had the same success rate in obtaining GCT cell line characterized by morphology, the gene expression Oct 4 and Nanog. But explants need a less time and had more cell than collagenase method. Viability of GCT cells decreased significantly after exposure to the clinical dose of H<sub>2</sub>O<sub>2</sub> 1% (p = 0,046), H<sub>2</sub>O<sub>2</sub> 3% (p = 0,043), and H<sub>2</sub>O<sub>2</sub> 5% (p = 0,043) for 10 minutes compared to controls. There was no significant difference for cell viability between 1%, 3% and 5% H<sub>2</sub>O<sub>2</sub> concentrations. While in in vitro doses (0,003%, 0,005%, 0,01%, 0,03%, 0,1%, 0,3%), 0.3% H<sub>2</sub>O<sub>2</sub> concentration for 5 minutes has the best effectivity in sterilizing GCT in vitro. There was a phenomenon of cell fixation after exposure of GCT cells to etanol in various concentrations, in which all cells die and its viability could not be analyzed. From the RT-PCR test it was found that there was a decrease in the expression of Oct 4 and Nanog genes along with an increase in the concentration of H<sub>2</sub>O<sub>2</sub> in vitro. Flow cytometry using Annexin V in conjunction with propidium iodide (PI) was dominated with PI marker detection which showed cell death due to necrosis, with the highest concentration amounted to 0.3%

**Conclusion** Explant was the best method for isolation and GCT cell culture. All of the cell from isolation and culture result had a characterization of GCT. Giving local a chemical adjuvants with clinical doses of H<sub>2</sub>O<sub>2</sub> concentrations of 1%, 3%, and 5% for 10 minutes in vitro had the same effectiveness in killing GCT cells. While the concentration of 0.3% H<sub>2</sub>O<sub>2</sub> for 5 minutes is the optimal therapy in GCT sterilization in vitro with necrosis cell death mechanism.