Isolasi dan kultur sel punca kanker osteosarkoma dengan metode sarcosphere = Isolation and culture of osteosarcoma cancer stem cell with sarcosphere method

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Abstrak

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Latar Belakang. Sel punca kanker (SPK) osteosarkoma didefinisikan sebagai sebagian kecil populasi sel osteosarkoma yang mempunyai kemampuan memperbaharui diri, menunjukan proliferasi dan mampu berdifferensiasi. SPK diduga bertanggung jawab terhadap resistensi kemoterapi, rekurensi dan metastasis. Studi ini bertujuan untuk melakukan isolasi, kultur dan karakterisasi secara in vitro SPK osteosarkoma manusia
Metode. Penelitian ini merupakan studi in vitro sebagai lanjutan yang memisahkan SPK osteosarkoma dari sel osteosarkoma manusia yang berhasil dikultur secara in vitro. Prosedur isolasi dan kultur SPK osteosarkoma dilakukan dengan metode sphere-forming assay pada ultra low well attachment surface plate. Setelah koloni sarcosphere terbentuk, dilakukan penanaman koloni tersebut pada tissue culture plate dan dilakukan karakterisasi pewarnaan Alizarin Red S, ekspresi penanda gen Nanog, Oct ¾, STAT3 dan CD133 dengan Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) dan ekspresi penanda protein alkali fosfatase, osteokalsin dan CD133 dengan Immunofluorescence Analysis (IFA).
Hasil. Dengan prosedur sphere forming assay dapat ditumbuhkan koloni sarcosphere yang berbentuk bulat, tiga dimensi serta tidak melekat pada substrat. Pada tissue culture plate didapatkan bentuk koloni sarcosphere berbentuk spindel dan melekat pada substrat. Pemeriksaan karakterisasi pewarnaan alizarin red s positif, ekspresi gen Nanog, Oct ¾ dan STAT3 yang dibuktikan dengan RT-PCR serta ekspresi protein alkali fosfatase, osteokalsin dan CD133 dengan metode IFA.
Simpulan. SPK osteosarkoma dapat diisolasi dan dikultur secara in vitro dari sel osteosarkoma manusia dengan metode sphere forming assay.
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ABSTRACT
Introduction. Osteosarcoma cancer stem cells (CSCs) are defined as a subpopulation of osteosarcoma cells which have the ability of self-renewal, proliferate and differentiate. CSCs may be responsible for chemotherapy resistance, recurrence and metastasis. This study aims to do isolation, culture and characterization of human osteosarcoma CSCs in vitro.
Method. This study was an in vitro study which extend the differentiation of osteosarcoma CSCs from human osteosarcoma cells that had been successfully cultured in vitro. Osteosarcoma CSCs had been isolated and cultured with sphere-forming assay method on an ultra low well attachment surface plate. After sarcosphere colonies formed, the planting of the colony on the tissue culture plate and Alizarin Red S staining characterization was performed, the expression of marker genes Nanog, Oct ¾, STAT3 and CD133 was obtained by Reverse Transcriptase Poslymerase Chain Reaction (RT-PCR) where as the expression of protein markers alkaline phosphatase, osteocalcin and CD133 was obtained by Immunofluorescence Analysis (IFA).

Result. Sphere-forming assay procedure could develop sarcosphere colonies which were rounded, three-dimensional and not attached to the subtsrate. In tissue culture plate, spindle-shaped sarcosphere colonies attached to the substrate. Alizarin Red S staining characterization was positive, the expression of Nanog, Oct ¾ and STAT3 gene was demonstrated by RT-PCR and protein expression of alkaline phosphatase, osteocalcin and CD133 was demostrated by IFA method.
Consclusion. CSCs in osteosarcoma can be isolated and cultured in vitro from human osteosarcoma cells by sphere-forming assay method.