

Intisari

Latar Belakang : Oksigen memiliki peranan penting dalam metabolisme tubuh. Penurunan kadar oksigen dapat menyebabkan hipoksia. Sel kanker yang mengalami hipoksia akan beradaptasi dengan mengekspresikan *Hypoxia Inducible factor 1* (HIF-1). HIF1 α berperan sebagai faktor transkripsi yang menginduksi ekspresi berbagai protein. Sebagian protein yang diekspresikan dapat disekresikan ke matriks ekstraseluler untuk mendukung pertumbuhan tumor. Penelitian ini mengidentifikasi protein di medium kultur yang disekresikan oleh sel tumor pada keadaan hipoksia dengan kromatografi cair - spektrofotometri massa (KC-SM).

Tujuan : Membandingkan profil protein yang disekresikan oleh galur sel kanker payudara T47D pada kondisi hipoksia dan normoksia. Mengidentifikasi protein spesifik sebagai kandidat protein penanda hipoksia pada galur sel kanker payudara T47D.

Metode : Galur sel kanker payudara T47D dikultur dalam kondisi normal hingga pasase dua sampai tiga. Sel dipanen dan dibagi menjadi 2 kelompok besar, yaitu normoksia dan hipoksia. Kelompok normoksia diinkubasi dengan kadar oksigen 20%, sedangkan kelompok hipoksia diinkubasi pada *hypoxic chamber* dengan konsentrasi oksigen 0,5% dan 5% selama 6 jam, 24 jam, dan 48 jam dengan medium tanpa serum. Protein pada medium kultur diisolasi dan dipresipitasi dengan trichloro acetic acid. Konsentrasi protein yang disekresi diukur dengan pemeriksaan *Bicinchoninic Assay* (BCA). Metode SDS-PAGE digunakan untuk visualisasi protein berupa pita-pita sesuai dengan berat molekul protein. Pita yang memiliki perbedaan antara kelompok hipoksia dan normoksia dianalisis lebih lanjut dengan KC-SM.

Hasil : Konsentrasi protein sekretorik pada kelompok hipoksia lebih tinggi daripada kelompok normoksia. Ditemukan gambaran pita protein pada kelompok hipoksia 0.5% dengan ukuran 50-75 kDa. Protein sekretorik yang berhasil diidentifikasi dengan KCKT-SM adalah keratin 1, 2, 9, dan 10.

Kesimpulan : Terdapat perbedaan ekspresi protein sekretorik pada kelompok hipoksia dan normoksia. Protein sekretorik yang teridentifikasi adalah keratin 1, 2, 9, dan 10. Protein tersebut disekresi oleh galur sel T47D dengan perlakuan hipoksia 0.5 %.

Kata Kunci : Hipoksia, protein sekretorik, kromatografi cair spektrofotometri massa

Abstract

Background : Oxygen has an important role in the body's metabolism. Decreased oxygen levels can cause hypoxia. Cancer cells that experience hypoxia will adapt by expressing Hypoxia Inducible factor 1 (HIF-1). HIF1 α acts as a transcription factor that induces the expression of various proteins. Some of the expressed proteins can be secreted into the extracellular matrix to support tumor growth. This study is an exploratory study to identify proteins in culture medium secreted by tumor cells under hypoxic conditions. Protein identification was carried out by liquid chromatography – mass spectrophotometry (LC-MS)

Objective : To compare the profile of the protein secreted by the T47D breast cancer cell line under hypoxic and normoxia conditions. Identify specific proteins as candidate hypoxia marker proteins in T47D breast cancer cell lines.

Methods : T47D breast cancer cell lines were cultured under normal conditions up to passages two to three. Cells were harvested and divided into 2 major groups, normoxia and hypoxia. The normoxia group was incubated under standard conditions, while the hypoxic group was incubated in a hypoxic chamber with 0.5% and 5% oxygen for 6 hours, 24 hours, and 48 hours with medium without serum. Proteins in the culture medium were isolated and precipitated with trichloro acetic acid. The concentration of secreted protein is measured by Bicinchoninic Assay (BCA) Protein then separated based on its molecular weight with SDS-page protein coupled with liquid chromatography-mass spectrophotometer for protein identification. The band that appeared only in the hypoxic group was further analyzed by LC-MS.

Result : The concentration of secretory proteins in the hypoxic group was higher than that in the normoxia group. Protein bands were found in the 0.5% hypoxia group with a size of 50-75 kDa. The secretory proteins identified by LC-MS are keratin 1, 2, 9, and 10.

Conclusion : There were differences in the expression of secretory proteins in the hypoxic and normoxia groups. The secretory proteins identified were keratin 1, 2, 9, and 10. These proteins were secreted by the T47D cell line with 0.5% hypoxia treatment.

Keywords : Hypoxia, secretory protein, liquid chromatography-mass spectrophotometer