Designation of CD24-CD44 breast cancer stem cell fraction pluripotency through C-MYC gene activity during hypoxic condition = Penunjukan pluripotensi fraksi sel punca kanker payudara CD24-/CD44 melalui aktivitas gen C-MYC selama kondisi hipoksia

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Abstrak

Latar belakang: Data dari World Health Organization, Centers for Disease Control and Prevention dan Kementerian Kesehatan RI menunjukan bahwa kanker payudara merupakan kanker yang paling umum pada wanita. Penyembuhan kanker melalui berbagai cara telah banyak dilakukan, namun pertumbuhan kembali dari kanker telah banyak dilaporkan. Sel punca kanker diyakini berperan dalam pertumbuhan kanker maupun rekurensi setelah pengobatan. Berdasarkan beberapa riset, CD44+/CD24- sel punca kanker memiliki potensial yang tinggi untuk menimbulkan kanker. Beberapa gen memiliki peran sebagai faktor transkripsi yang berkontribusi dalam pertumbuhan kanker dan beberapa berperan juga dalam mempertahankan tingkat pluripotensi kanker. c-Myc merupakan salah satu gen yang mempertahankan iPS (induced pluripotent stem cells) bersama dengan KLF4, Oct4, SOX2 dan Nanog. Namun demikian, selama pertumbuhan kanker, lingkungan mikro dari kanker menjadi hipoksia. Berhubungan dengan ini, pengaruh hipoksia terhadap ekspresi gen yang berfungsi dalam pluripotensi masih belum jelas. Oleh karena itu, eksperimen ini menyelidiki ekspresi gen c-Myc dalam sel punca kanker yang diinduksi hipoksia.
Metode: Sel punca kanker payudara CD44+/CD24- diinduksi oleh beberapa durasi hipoksia (0 jam, 0.5 jam, 4 jam, 6 jam dan 24 jam). Total RNA sel kemudian diekstraksi dan mRNA gen c-Myc diamplifikasi melalui one-step qRT-PCR. Ekspresi relative dari gen c-Myc dilakukan dengan formula Livak berdasarkan nilai Ct yang diperoleh dengan gen 18S. Sebagai kontrol, konfirmasi ekspresi gen c-Myc dikonfirmasi melalui elektroforesis.
Hasil: Ekspresi c-Myc pada sampel sel punca kanker payudara CD44+/CD24- yang diinduksi hipoksia selama 0.5 jam sedikit mengalami peningkatan dibandingkan dengan sampel 0 jam walaupun tidak signifikan. Ekspresi c-Myc pada sampel yang diinduksi hipoksia selama 4, 6 dan 24 jam menurun dibandingkan sampel yang tidak diinduksi hipoksia.
Kesimpulan: Ekspresi c-Myc pada sel punca kanker CD44+/CD24- yang digunakan dalam eksperimen ini cenderung menurun pada 3 durasi hipoksia yang berbeda (4 jam, 6 jam dan 24 jam) pada kondisi in vitro.
.....Background: Data from World Health Organization, Centers for Disease Control and Prevention and Kementerian Kesehatan RI show that breast cancer is the most common cancer among women. Eradicating cancer through several treatments have been done but there are cases in which cancer relapse is reported. Cancer stem cells have been found to develop the cancer as well as play important role in cancer regrowth. According to some researches, CD44+/CD24- breast cancer stem cells potential to cancer development is high. Several genes which have role as transcription factors may contribute to cancer growth and some act to maintain the cancer stemness and pluripotency level. c-Myc is one gene which maintains iPS (induced pluripotent stem cells) along with KLF4, Oct4, SOX2 and Nanog. However, during the cancer growth the cancer microenvironment becomes hypoxic. In accordance to this, the effect of hypoxia towards the gene expression acting in cancer pluripotency was not yet clear. Therefore c-Myc expression in hypoxia-induced

breast cancer stem cells was assessed in this research.
Method: The CD44+/CD24- breast cancer stem cells (BCSCs) are induced by several hypoxia durations (0 hour, 0.5 hour, 4 hours, 6 hours and 24 hours) in hypoxia chamber. The mRNA of BCSCs is extracted through RNA isolation procedure. Following this, qRT-PCR procedure is done to amplify the mRNA. The Ct (cycle threshold) obtained from qRT-PCR are calculated using Livak formula to get the c-Myc relative expression from the samples. Ct of 18S is used to normalize the c-Myc Ct. Electrophoresis is done next to confirm the c-Myc expression.
Results: c-Myc expression in 0.5 hour hypoxia induced CD44+/CD24- breast cancer stem cells sample is slightly high than in 0 hour hypoxia induced sample, even though the increase is not significant. Meanwhile, c-Myc expression in 4, 6 and 24 hours hypoxia induced samples are lower than 0 hour hypoxia induced sample.
Conclusion: c-Myc expression from the breast cancer stem cell CD44+/CD24- samples used in this experiment tend to have gradual decrease during 3 different periods (0 hour, 0.5 hour, 4 hours, 6 hours and 24 hours) of hypoxia in vitro.