Efek Pemaparan Tamoksifen Berulang pada Sel Punca Kanker Payudara CD24-/CD44+: Kajian mengenai Sensitivitas Terapi melalui Perubahan Ekspresi Estrogen Reseptor Alfa dan Transporter Efluks Multidrug Resistance-Associated Protein 2 (MRP2) = The effects of repeated tamoxifen exposure to the breast cancer stem cells CD24-/CD44+: A study on sensitivity to therapy through changes in expression of estrogen receptor alpha and efflux transporter Multidrug Resistance-Associated Protein 2 (MRP2)

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Abstrak

Latar Belakang: Sel punca kanker merupakan populasi sel minor yang memiliki kemampuan self-renewal dan proliferasi tak terbatas sehingga bersifat tumorigenik dan diduga berperan dalam penurunan sensitivitas terhadap berbagai terapi kanker. Tamoksifen merupakan terapi lini pertama pada kanker payudara ER positif namun penggunaan jangka panjangnya menimbulkan masalah resistensi. Beberapa faktor yang diduga berperan dalam penurunan sensitivitas sel terhadap Tamoksifen yakni modulasi pensinyalan estrogen melalui ER?66; dan ER?36 (yang diketahui memperantarai pensinyalan non-genomik), serta ekspresi transporter effluks seperti MRP2 yang berperan dalam penurunan kadar Tamoksifen intraseluler. Penelitian ini bertujuan untuk menganalisis efek pemaparan Tamoksifen berulang pada sel punca kanker payudara CD24-/CD44+, dalam kaitannya mengenai sensitivitas terapi melalui perubahan ekspresi estrogen reseptor alfa dan transporter efluks MRP2.

Metode: Selpunca kanker payudara CD24-/CD44+ dipaparkan Tamoksifen 1 ?M selama 21 hari dengan DMSO sebagai kontrol negatif. Viabilitas sel setelah pemaparan Tamoksifen diuji dengan metode trypan blue exclusion. Sifat tumorigenik sel setelah pemaparan (CD24-/CD44+(T)) diuji dengan mammossphere formation assay dan dibandingkan dengan sel CD24-/CD44+(0) yang belum dipaparkan Tamoksifen. Ekspresi mRNA Oct4, c-Myc, ER?66, ER?36 dan MRP2 dianalisis dengan one step quantitative RT-PCR.

Hasil: Terjadi penurunan sensitivitas sel punca kanker payudara CD24-/CD44+(T) yang dipaparkan Tamoksifen selama 21 hari yang ditunjukkan dengan kenaikan viabilitas sel hingga 125,2%. Tamoksifen tidak dapat menekan sifat tumorigenik sel CD24-/CD44+(T) yang dibuktikan melalui jumlah mammosfer yang tidak berbeda bermakna dibandingkan dengan CD24-/CD44+(0). Penurunan sensitivitas sel CD24-/CD44+(T) juga dibuktikan melalui peningkatan ekspresi Oct4 dan c-Myc; keduanya merupakan petanda pluripotensi dan c-Myc juga dikenal sebagai petanda keganasan. Parameter penurunan sensitivitas seperti ER?66, ER?36 dan MRP2 juga menunjukkan peningkatan ekspresi pada hari ke-15 namun menurun kembali pada hari ke-21 yang menunjukkan adanya mekanisme regulasi lain yang mungkin terlibat dalam penurunan sensitivitas sel punca kanker payudara terhadap Tamoksifen.

Kesimpulan: Pemaparan Tamoksifen berulang dapat menurunkan sensitivitas sel punca kanker payudara CD24-/CD44+ melalui perubahan ekspresi estrogen reseptor alfa dan transporter efluks MRP2.

.....Background: Cancer stem cells are minor population of cells possessing self-renewal and unlimited proliferation abilities which support their tumorigenicity and role in decreased sensitivity to many cancer therapies. Tamoxifen is a first line therapy for breast cancer patients with positive ER status. Nonetheless, after 5 years of its long term use eventually leads to recurrence and resistance in 50% of patients receiving tamoxifen therapy. Among some factors that might play role in decreased sensitivity to tamoxifen are modulation of estrogen signaling through ER?66 and ER?36 (the latter known for its non-genomic estrogen signaling), and expression of efflux transporter such as MRP2 responsible for decreased intracellular tamoxifen level. The objective of this study is to analyze the effects of long term tamoxifen exposure toward decreased sensitivity of the breast cancer stem cells CD24-/CD44+ through changes in expression of estrogen receptor alpha and efflux transporter MRP2.

Methods: Breast cancer stem cells CD24-/CD44+ were exposed to 1 ?M tamoxifen for 21 days with DMSO as negative control. After exposure with 1 ?M tamoxifen, the cell viability were tested by the trypan blue exclusion method. Cell tumorigenicity of tamoxifen-exposed CD24-/CD44+(T) and CD24-/CD44+(0) (before treatment) were tested by the mammosphere formation assay. The expression of Oct4, c-Myc, ER?66, ER?36 andMRP2 mRNAs were analyzed by one step quantiative RT-PCR.

Results: A decreased sensitivity of the breast cancer stem cells CD24-/CD44+ exposed with 1 ?M tamoxifen for 21 days was observed as indicated by an increased cell viability up to 125.2%. In the presence of tamoxifen, breast cancer stem cells CD24-/CD44+(T) exhibited tumorigenic properties as indicated in no significant difference in the formation of mammosphere unit compared to those of CD24-/CD44+(0). After exposure with 1 ?M tamoxifen for 21 days, an elevated level of Oct4 and c-Myc expressions were observed; both are known as pluripotency markers and the latter also known as marker of aggresiveness. Parameters for a decreased sensitivity such as ER?66, ER?36 and MRP2 also exhibited an elevated expression after 15 days of exposure, but the decreased expression after 21 days of exposure suggests that there might be another mechanism involved in decreased sensitivity of the breast cancer stem cells toward tamoxifen.

Conclusion: Long term tamoxifen exposure may decrease the sensitivity of the breast cancer stem cells CD24-/CD44+ through changes in expression of estrogen receptor alpha and efflux transporter MRP2.