Pengaruh Pemberian TGF-1 Rekombinan Manusia pada Sel Punca Kanker Payudara ALDH+ terhadap Ekspresi Marker Kepuncaan Melalui Pensinyalan Autokrin TGF-1 = The Impact of Human TGF-1 Recombinant Protein Treatment to BCSC ALDH+ on The Expression of Stemness Marker Through TGF-1 Autocrine Signaling

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Abstrak

Latar Belakang: Breast Cancer Stem Cells (BCSC) merupakan populasi sel kanker payudara yang mempunyai sifat sel punca. BCSC menjaga stabilitas tumor dengan menginisiasi pembentukan populasi sel kanker baru serta memberikan kekebalan terhadap terapi. BCSC dapat berinteraksi dengan lingkungan mikro tumor yang melepaskan berbagai sitokin dan growth factor, termasuk TGF-1. Melalui mekanisme autoinduksi, TGF-1 dapat meningkatkan produksi TGF-1 endogen dan pensinyalan autokrinnya. Pensinyalan autokrin TGF-1 dapat meningkatkan pengaruh tumor promotor TGF-1 dalam perkembangan kanker melalui penguatan karakter kepuncaan dan sifat tumorigenik. Penelitian ini bertujuan untuk enganalisis efek pemberian TGF-1 rekombinan manusia pada sel punca kanker payudara (aldehyde dehydrogenase positive, ALDH+) terhadap ekspresi marker kepuncaan melalui pensinyalan autokrin TGF-1. Sebagai pembanding digunakan kanker payudara subtipe triple negative (TNBC).
Metode: BCSCs manusia (ALDH+) dan TNBC (MDA-MB-231) dikultur dalam Dulbecco's Modified Eagle Medium/Nutrient Mixture F12/HG (DMEM F12/HG) dengan suplemen 0,1 ng/ml protein rekombinan TGF-1 manusia (rhTGF-1) selama periode 1, 2 dan 4 jam. Medium kultur kemudian diganti dengan DMEM F12/HG tanpa serum selama 24 jam. Tingkat ekspresi mRNA reseptor TGF- tipe 1 (TR1), TGF-1, faktor transkripsi pengikat oktamer 4 (OCT4), dan anggota A1 keluarga aldehida dehidrogenase 1 (ALDH1A1) dianalisis menggunakan real time reverse transcriptase polymerase chain reactions (RT-qPCR). Kadar protein TGF-1 dalam media kultur ditentukan dengan menggunakan enzyme-linked immunosorbent assay (ELISA). Sifat tumorigenik sel diuji dengan uji mammosphere forming unit (MFU assay).
Hasil: Tingkat ekspresi mRNA dan protein TGF-1 BCSC setelah perlakuan tampak meningkat namun tidak pada TNBC. mRNA TR1 BCSCs meningkat pada periode perlakuan 1 dan 2 jam, sedangkan pada TNBC hanya pada periode 1 jam. Penanda kepuncaan ALDH1A1 dan OCT4 tampak meningkat pada BCSC namun tidak pada TNBC. Uji MFU menunjukkan sifat tumorigenik kedua kelompok sel terutama pada periode perlakuan 2 jam tampak meningkat.
Kesimpulan: Perlakuan TGF-1 dalam konsentrasi rendah dan dalam waktu singkat memicu autoinduksi pada BCSCs yang menyebabkan peningkatan ekspresi

gen kepuncaan melalui pensinyalan autokrin. Sedangkan pada TNBC, peningkatan ekspresi marker kepuncaan tidak terjadi. Namun demikian, sifat tumorigenik BCSC dan TNBC tetap meningkat.
.....Background: Breast Cancer Stem Cells (BCSC) is a population of breast cancer cells that have stem cell characteristics. BCSCs maintain tumor stability by initiating the formation of new cancer cell populations and providing resistance to therapy. BCSCs can interact with the tumor microenvironment which releasing various cytokines and growth factors, including TGF-1. Through the autoinduction mechanism, TGF-1 can increase endogenous TGF-1 production and autocrine signaling. TGF-1 autocrine signaling can increase the tumor promoter role of TGF-1 in cancer development by enhancing the stemness and tumorigenic properties. This study aims to analyze the effect of Human TGF-1 recombinant protein treatment to breast cancer stem cells (aldehyde dehydrogenase positive, ALDH+) on the expression of stemness marker through TGF-1 autocrine signaling. Triple negative breast cancer (TNBC) was used as a comparison.
Methods: Human BCSCs (ALDH+) and TNBC (MDA-MB-231) were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F12/HG (DMEM F12/HG) with 0.1 ng / ml recombinant protein of human TGF-1 supplementation (rhTGF-1) over 1, 2 and 4 hour periods. The culture medium was then replaced with DMEM F12/HG serum-free for 24 hours. The expression levels of the TGF-receptor type 1 (TR1), TGF-1, octamer-binding transcription factor 4 (OCT4), and members of the A1 family of aldehyde dehydrogenase 1 (ALDH1A1) mRNA were analyzed using real time reverse transcriptase polymerase chain reactions (RT-qPCR). TGF-1 protein levels in conditioned medium were determined using an enzyme-linked immunosorbent assay (ELISA). The tumorigenic properties of cells were tested by the mammosphere forming unit (MFU) assay.
Results: The expression level of mRNA and TGF-1 BCSC protein after treatment appeared to be increased but not in TNBC. mRNA TR1 BCSCs increased in the treatment period of 1 and 2 hours, whereas in TNBC only in the 1 hour period. The markers of ALDH1A1 and OCT4 expression appeared to be increased in BCSC but not in TNBC. The MFU test showed that the tumorigenic properties of both cell groups, especially in the 2 hour treatment period, appeared to be increasing.
Conclusion: Treatment of TGF-1 in low concentrations and in a short time triggered autoinduction of BCSCs and leads to the increased expression of stemness genes marker via autocrine signaling. Whereas in TNBC, this increase in the expression of the stemness markers did not occur. However, the tumorigenic nature of BCSC and TNBC continues to increase.