

Sekresi Sitokin IL-6 pada Asidifikasi Ekstrasel PBMC dan Dampaknya terhadap Pensinyalan Kepuncean IL-6-STAT3 pada Sel Punca Kanker Payudara Manusia = Secretion of IL-6 cytokine from extracellular acidified PBMC and its impact on IL-6-STAT3 stemness signaling of human breast cancer stem cells

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Abstrak

Latar Belakang: Studi dalam bidang kanker bukan hanya fokus pada sel kanker namun juga pada lingkungan sel kanker serta interaksi yang terjadi antara sel kanker dengan lingkungan. Salah satu faktor dalam lingkungan sel kanker yang turut berperan adalah SASP (senescence associated secretory phenotype) yang disekresikan oleh sel yang mengalami penuaan. Hingga saat ini, belum diketahui efek faktor SASP yang disekresikan oleh peripheral blood mononuclear cell (PBMC) yang telah dipaparkan dengan kondisi ekstrasel yang asam (asidifikasi) terhadap kepuncean BCSC terkait pensinyalan IL6-STAT3.

Metode: BCSC dikultur dengan 10% CM-PBMC pH 7,4 dan 6,6 (dari 20 sampel) dalam medium DMEM/F-12 (kondisi standar selama 24 jam). IL-6 pada CM- PBMC dianalisis menggunakan Luminex dilanjutkan dengan konfirmasi menggunakan ELISA. Ekspresi protein pgp130, gp130, pSTAT3, STAT3, dan OCT4 dianalisis menggunakan metode Western Blot. Ekspresi relatif mRNA OCT4 dianalisis menggunakan qRT-PCR (perhitungan relatif Livak) dengan 18s sebagai house-keeping gene. Jumlah sel hidup dianalisis menggunakan metode ekslusi trypan blue. Pembentukan mamosfer dianalisis dengan metode MFU.

Hasil: Kadar IL-6 secara signifikan lebih tinggi pada CM-PBMC pH 6,6 dibandingkan pH 7,4. Tidak terdapat perbedaan secara signifikan pada ekspresi protein pgp130, gp130, pgp130/gp13, pSTAT3, STAT3, dan pSTAT3/STAT3 pada pemberian CM-PBMC pH 7,4 dan 6,6. Ekspresi mRNA OCT4 dan protein OCT4 lebih tinggi pada pemberian CM-PBMC pH 6,6 dibandingkan pH 7,4 namun tidak signifikan. Jumlah sel hidup dan pembentukan mamosfer secara signifikan lebih tinggi pada pemberian CM-PBMC pH 6,6 dibandingkan pH 7,4. Korelasi positif terjadi antara rasio IL-6 dengan rasio jumlah sel hidup. Pada kelompok IL-6 meningkat, terdapat korelasi positif antara rasio IL-6 dengan rasio mRNA OCT4 dan rasio pembentukan mamosfer. Selain itu, terdapat korelasi positif antara rasio mRNA OCT4 dan protein OCT4. Kesimpulan : Sekresi sitokin IL-6 pada asidifikasi ekstrasel peripheral blood mononuclear cells dapat meningkatkan ekspresi OCT4 dan pembentukan mamosfer walaupun tidak terbukti mengaktifkan pensinyalan IL-6 STAT3.

.....Background: In cancer study, focus of the study is not only on cancer cells but also on the environment of cancer cells and the interactions between cancer cells and the environment. One of the factors in the cancer cell environment that played a role is SASP (senescence associated secretory phenotype) secreted by senescence cells. It was not known the effect of SASP factor secreted by peripheral blood mononuclear cells (PBMC) which has been exposed to acidic extracellular conditions to induce aging, on the BCSC stemness. Methods: BCSCs were cultured with 10% CM-PBMC pH 7.4 and 6.6 (from 20 samples) in DMEM/F-12 adjusted to 7.4 under standard conditions for 24 hours. IL-6 on CM-PBMC was analyzed using Luminex and ELISA. The pgp130, gp130, pSTAT3, STAT3, and OCT4 protein expressions were analyzed using the Western Blot method with -actin as a house-keeping protein. Relative expression of OCT4 mRNA was

analyzed using qRT-PCR (relative Livak calculation) with 18s as the house-keeping gene. The number of living cells was analyzed using the trypan blue exclusion method. The formation of the mammosphere was analyzed using the MFU method.

Results: The IL-6 concentration increased at CM-PBMC pH 6.6 compared to pH 7.4. There was no difference in the expression of gp130, gp130, gp130 / gp130, pSTAT3, STAT3, and pSTAT3 / STAT3 protein expression with the aministration CM-PBMC pH 7.4 and pH 6.6. There was insignificant increase in OCT4 mRNA and OCT4 protein expression on CM-PBMC pH 6.6 compared to pH 7.4. The number of BCSC living cells and mammosphere formation increase with the aministration CM-PBMC pH 6.6 compared to pH 7.4. There were positive correlation between IL-6 ratio with number of live cell ratio. In, upregulated IL-6 secretion group, there were positive correlation between IL-6 ratio with mRNA OCT4 ratio and mammosphere formation ratio. Furthermore, there was a positive correlation between OCT4 mRNA ratio with OCT4 protein ratio.

Conclusion: Secretion of IL-6 cytokines from acidified peripheral blood mononuclear cells increase OCT4 expression and mammosphere formation although it has not been shown to activate IL-6 STAT3 signaling.