

INTISARI

Latar Belakang Karsinoma nasofaring (KNF) merupakan kanker kepala dan leher dengan prevalensi tinggi pada daerah endemik, termasuk Indonesia. Perkembangan KNF berhubungan erat dengan infeksi virus Epstein-Barr (EBV) dan inflamasi. *EBV encoded RNAs* (EBER) merupakan transkrip virus yang dapat berinteraksi dengan TLR3 dan menginduksi sitokin pro-inflamasi, berujung pada perekrutan sel-sel imun ke lingkungan mikro tumor.

Tujuan Penelitian ini bertujuan untuk mengetahui hubungan ekspresi TLR3 dan sitokin pro-inflamasi (TNF α , IL6) terhadap distribusi macrophagocytus M2 (M2) dan cellulae T regulatrices (Treg) pada jaringan KNF yang mengekspresikan EBER.

Metode Dua puluh tiga sampel irisan jaringan *formalin-fixed paraffin-embedded* (FFPE) pasien KNF dari tahun 2008-2010 yang mengekspresikan EBER digunakan dalam penelitian ini. Ekspresi TLR3, TNF α dan IL6 diperiksa menggunakan metode *immunofluorescence assay* (IFA). Distribusi macrophagocytus M2 dan cellulae T regulatrices diperiksa menggunakan metode imunohistokimia dengan antibodi anti-CD163 dan anti-FOXP3 secara berurutan. Kuantifikasi intensitas fluoresen dilakukan dengan perangkat lunak imageJ menggunakan metode *red green blue space* (RGB). Nilai M2 diinterpretasi dengan metode *eye balling*, dibagi menjadi skor 0 (negatif), 1 (sedikit), 2 (*focal*), 3 (banyak). Nilai Treg diambil dari rerata jumlah sel positif dari 5 lapang pandang. Penilaian M2 dan Treg dilakukan pada perbesaran 40x. Hubungan antar variabel diuji dengan uji korelasi Spearman, nilai r digunakan untuk melihat besarnya korelasi antar parameter.

Hasil Semua spesimen KNF (EBER⁺) menunjukkan ekspresi TLR3 yang terletak di intraseluler. Ekspresi TNF α dan IL6 keduanya disekresikan pada area ekstraseluler, namun TNF α juga tereksresi pada membran sel. Ekspresi TNF α lebih tinggi 2x lipat dibandingkan IL6. Mayoritas spesimen memiliki distribusi M2 dengan skor rendah (56,52%) dan Treg kategori tinggi (52,17%). TLR3 dan IL6 memiliki hubungan sebesar 12,9%, sedangkan 87,1% dijelaskan oleh faktor lain. TNF α berkorelasi positif dengan distribusi M2 sebesar 13,7% dan dengan distribusi Treg sebesar 12,9%, sedangkan sisanya dijelaskan oleh faktor lain.

Kesimpulan Ekspresi TLR3 memiliki korelasi negatif dengan TNF α , distribusi M2 dan Treg, serta korelasi positif dengan IL6. Ekspresi TNF α memiliki korelasi positif dengan distribusi M2 dan Treg. Ekspresi IL6 memiliki korelasi negatif dengan distribusi M2 dan Treg pada jaringan KNF EBER⁺.

Kata kunci EBER, TLR3, sitokin pro-inflamasi, M2, Treg

ABSTRACT

Background Nasopharyngeal Carcinoma (NPC) is the most common head and neck cancer with high prevalence among native population in Indonesia. Development and the progress of NPC are closely associated with Epstein-Barr Virus infection and inflammation. EBV encoded RNAs (EBER) is the most abundant viral transcript which could be recognized by Toll-like receptor 3 (TLR3) and induce tumor cells to produce cytokines, in which macrophages are recruited and activated, leading to a favorable microenvironment for solid tumor growth.

Objectives The aim of this study was to investigate the correlation between the expression of TLR3 and pro-inflammatory cytokines (TNF α , IL6) with the distribution of macrophagocytus M2 dan cellulae T regulatrices (Treg) on EBER⁺ NPC tissues.

Methods A total of 23 samples FFPE tissues obtained from NPC patients from 2008-2010 which expressed EBER was collected. The expression of TLR3, TNF α and IL6 were examined using immunofluorescence assay (IFA). The distribution of macrophagocytus M2 and cellulae T regulatrices were examined by immunohistochemistry with anti-CD163 and -FOXP3 antibodies, respectively. Fluorescence intensity quantification analysed with RGB space method using ImageJ software. The M2 interpretation was done by eye balling method and the M2 score divided into 0 (negative), 1 (scant), 2 (focal), 3 (abundant). The average number of Treg FOXP3⁺ cells in 5 high power fields was count. The relationship between variables were tested by Spearman correlation test, the coefficient correlation was used to see the correlation between variables.

Results All EBER⁺ NPC specimens showed TLR3 expression intracellularly. The expression of TNF α and IL6 were secreted to the extracellular area, and TNF α was also expressed on cell membrane. The expression of TNF α was 2 times higher than IL6. Most specimens showed low M2 score (56,52%) and high Treg (52,17%). The correlation between TLR3 and IL6 was 12,9%, while 87,1% related to other factors. TNF α has a positive correlation with M2 distribution of 13,7% and Treg distribution of 12,9%, while the rest is explained by other factors.

Conclusions The expression of TLR3 was negatively correlated with TNF α , the distribution of M2 and Treg, while positively correlated with IL6. The expression of TNF α was positively correlated with the distribution of M2 and Treg. The expression of IL6 was negatively correlated with the distribution of M2 and Treg in EBER⁺ NPC tissues.

Keywords EBER, TLR3, pro-inflammatory cytokines, M2, Treg