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# Multiplex immunohistochemistry for immune profiling of HPV-associated head and neck cancer

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Human Papilloma Virus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) is clinically distinct from HPV-negative HNSCC, and as such requires differential therapeutic approaches. Accumulating evidence indicates a significant linkage between the immune response within the tissue and pathogenesis of HPV-associated HNSCC. To further elucidate immune-related signatures in HPV-associated HNSCC, we performed multiplex histological analysis in de-identified tissue microarray sections including HPV-positive (n = 21), HPV-negative (n = 17), and normal oropharynx (n = 8). Following immunohistochemistry (IHC) for CD45, CD3, CD8, Foxp3, T-bet, GATA-3, RORgT, CD20, CD56, CD68, MHC class II, CSF1R, CD66b, tryptase, CD83, DC-SIGN, PD-1, and PD-L1, the cell intensity per $mm^2$ ratio/composition, localization were quantitatively evaluated. The HPV-status was confirmed by HPV16/18 polymerase chain reaction and IHC for $p16^{INK4a}$. IHC for p16 or EpCAM were utilized for defining tumor region. Infiltration of T cell populations including $CD45^+CD3^+CD8^+$ T cells ($P < 0.01$), $CD45^+CD3^+CD8^-Foxp3^+$ regulatory T cells ($P < 0.05$) and $CD45^+CD3^+CD8^-Foxp3^+T\text{-}bet^+$ Th1 cells ($P < 0.01$), $CD45^+CD20^+CD3^-$B cells ($P < 0.05$), $CD45^+CD68^+CD163^-$ macrophages ($P < 0.001$), and $CD45^+Tryptase^+$ mast cells ($P < 0.01$) was significantly higher in the HPV-positive group than in the HPV-negative group. CD8/CD68 ratio of HPV-positive tumor was higher than that of HPV-negative tumor ($P < 0.05$), and the highest $CD163^-CD68^+/CD163^+CD68^+$ ratio was observed in the intra-tumor region of HPV-positive tumors. High PD-L1 expression on $CD68^+CD163^+$ macrophages and MHC class $II^+CD83^+$ dendritic cells was intensively observed in the intra-tumor region of the HPV-positive group while maturation of dendritic cells assessed by CD83/DC-SIGN ratio was significantly higher in the peritumoral stroma than intra-tumor regions ($P < 0.05$), indicating distinct immune regulatory mechanisms between intra and peritumoral regions. These signatures provide further evidence of anti-tumor immunity against HPV-positive head and neck cancer, and potentially lead to personalized treatment including immunomodulatory therapeutic targets specialized for the HPV/immune status.

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