り小胞体ストレスの評価を、Annexin V/PI 染色細胞のフローサイトメトリーおよび、ギムザ染色による形態観察により細胞死の評価を行った。
＜結果・考察＞　A549 細胞において、FTY は Lap および Sor の殺細胞効果を顕著に増強したが、Dab および Vem との併用効果は小さかった。FTY と Lap および Sor との併用効果は、膵癌細胞株 BxPC-3 および PANC-1 でも同様に観察され、特に BxPC-3 細胞における殺細胞効果の増強は、カスパーゼ阻害剤 z-VAD-fmk を添加することで抑制された。さらに BxPC-3 細胞では、FTY と Lap との併用によって、Annexin V 陽性細胞数の増加およびアポトーシス特有の核の断片化、また、小胞体ストレス応答遺伝子の発現増加および小胞体の膨化が観察された。以上のことから、FTY と Lap との併用は、小胞体ストレス負荷の増大を介して、BxPC-3 細胞のアポトーシス様細胞死を誘導することが示唆された。

5.

Programmed cell death 2 forms coinhibitory microclusters that directly attenuate T cell receptor signaling by recruiting phosphatase SHP2

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Background: Anti-PD-1 antibodies have made tremendous therapeutic effects on advanced or recurrent non-small cell lung cancer. However, the expression level of PD-L1 in tumor tissue which is the biomarker in the clinical setting is not necessarily correlated with their efficacy. In recent reports, some kinds of tumors express PD-L2 which is another ligand of PD-1. It is possible that the binding between PD-L2 and PD-1 is contributing to this mechanism. Because we intended to analyze the microstructural basis of immunological synapse, we used a system of a planar bilayer incorporated with glycophosphatidylinositol（GPI）-anchored intercellular adhesion molecule 1（ICAM-1）and major histocompatibility complex（MHC）class II（I-Ek）loaded with a moth cytochrome c（MCC）peptide. Using our unique imaging analysis, we found the “TCR microcluster”, where TCR proximal signaling molecules are recruited. Here, we investigated the PD-1-PD-L2 pathway and PD-L1 vs PD-L2 competition toward PD-1 binding using this dynamic imaging technique.
Methods: We established tumor cell line（BHK）highly expressing murine PD-L2（mPD-L2）-GPI and purified mPD-L2-GPI by affinity column with anti-PD-L2. Primary CD4+ T cell isolated from AND-Tg mice in Rag2－/－ Pdcd1－/－ background and T cell hybridoma expressing AND-TCR（2D12）were retrovirally transduced with PD-1-GFP or PD-1-HaloTag and GFP-SHP1/2. We used planar bilayers of mPD-L2-GPI, mICAM-1 and I-Ek loaded with MCC peptide and observed different kinds of T cell by confocal microscopy.
Results: We showed PD-1 is translocated to TCR microclusters and then accumulates at the central region of the immunological synapse in the presence of PD-L2. We also confirmed the rapid and transient recruitment of SHP2, not SHP1, to PD-1 microclusters by PD-1-PD-L2 binding. Biochemical assays demonstrated that PD-L2 dephosphorylates TCR downstream signaling molecules, resulting in the reduction of IL-2 production by forming PD-1-PD-L2 microclusters. In the presence of both PD-L1 and PD-L2, PD-L2 accumulations toward PD-1 microclusters are stronger than those of PD-L1. We also confirmed the correlation between imaging results and IL-2 production.
Conclusions: Our results indicate that the function of PD-L1 and PD-L2 is the same in the point of suppressing TCR signaling by the recruitment of SHP2 for the initiation of T cell activation. However, PD-L2 more potently occupy PD-1 microclusters than PD-L1. We are now evaluating the effect of anti-PD-1/L1/L2 antibody for TCR signaling.