Targeting the microtubule-network rescues CTL killing efficiency in dense 3D matrices

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Dense three-dimensional (3D) matrix, a prominent physical feature of the solid tumor environments, is one of the potential reasons to impair immune surveillance against tumor cells. Cytotoxic T lymphocytes (CTLs) are one of the key players to eliminate tumor cells. Here, using the real-time 3D killing assay, we found that the killing efficiency of primary human CTLs was substantially impaired in dense collagen matrices (4 mg/ml and 5mg/ml) compared to less dense collagen matrix (2 mg/ml). Although dense collagen did not significantly affect the expression of cytotoxic proteins in CTLs, dense collagen impaired CTL motility, leading to decreased searching efficiency of CTLs. Furthermore, we identified that two physical features of dense collagen matrices, high stiffness and small pore size, contributed to impaired CTL motility and reduced killing efficiency. Notably, in the 3D collagen matrix, CTL migration velocity was positively correlated with nucleus deformability. In dense matrices, microtubule disruption with nocodazole led to enhancement in nucleus deformability, CTL migration, searching efficiency, and killing efficiency. Moreover, treating CTLs with vinblastine, which is a chemotherapy drug targeting microtubules, rescued impaired CTL killing efficiency in dense matrices. Our findings suggest targeting the microtubule network as a promising strategy to enhance the efficacy of CTL-based immunotherapy against solid tumors.