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Hydroxychloroquine increases the tumor killing efficiency via elevating the membrane MHC-I protein levels of tumor cells

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Background:

Hydroxychloroquine (HCQ) was firstly developed as an antimalarial drug, while recently it has been used to treat various types of cancer due to its molecular mechanism in inhibiting autophagy process, induction of apoptosis, and the inhibition of CXCL12/CXCR4 pathway in cancer cells. Previous studies found that autophagy promotes immune evasion of pancreatic cancer by degrading major histocompatibility complex class I (MHC-I), so we supposed HCQ treatment might restore or even elevate the MHC-I levels of tumor cells through inhibiting autophagy and then benefit tumor infiltrating lymphocytes (TILs) immunotherapy.

Methods:

Malignant melanoma cell line A-375, non-small cell lung cancer (NSCLC) cell line NCI-H2122, ovarian cancer OVCAR-8 and glioblastoma U-87 MG were subjected to HCQ treatment for 48 hours and the protein levels of membrane MHC-I and programmed cell-death ligand 1 (PD-L1) were measured by flow cytometry. IFN-γ was set as a positive control for its well-known function in elevating MHC-I levels. Western blots were carried out to verify the autophagy inhibition. Real-time cell analysis (RTCA) was used to evaluate the in vitro cytotoxicity for transgenic hmTCRT cells specifically recognizing NY-ESO-1 epitope and targeting HCQ treated or non-treated NY-ESO-1 positive A-375 cells. Using the same concentrations as treating tumor cells, 15μM, 20μM HCQ treatment was carried out on TILs in rapid expansion phase to determine whether their proliferation would be affected.

Results:

HCQ treatment can significantly elevate the protein levels of membrane MHC-I for tumor cell lines A-375, NCI-H2122, OVCAR-8 and U-87 MG (Figure 1A-1D, all P values of t-test <0.05), and the elevation can sustain for 72 hours after removing HCQ from the culture system (Figure 1E-1H, all P values of t-test <0.05).

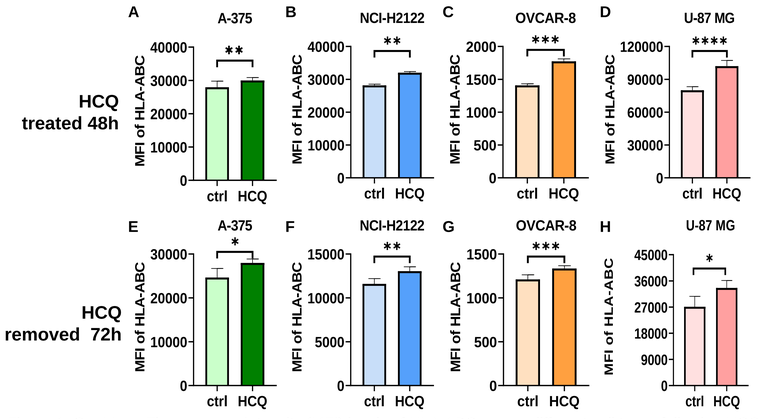


Figure 1. GeoMean Fluorescence Intensity (MFI) levels of HLA-ABC across different cell types following HCQ stimulation. (A–D), MFI values measured 48 hours post-HCQ stimulation in distinct cell populations; (E-H), MFI values recorded 72 hours after HCQ withdrawal (i.e., 48 hours post-stimulation + 72 hours recovery). Data are presented as mean ± SD (error bars) from three independent experiments. *, p < 0.05; **, p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

Surface PD-L1 expression in the four cell lines remained unaltered following HCQ treatment when compared to the DPBS control group. In contrast, IFN-γ treatment induced a significant upregulation of PD-L1 levels (Figure 2).

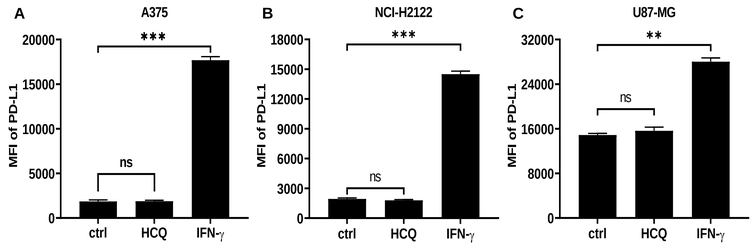
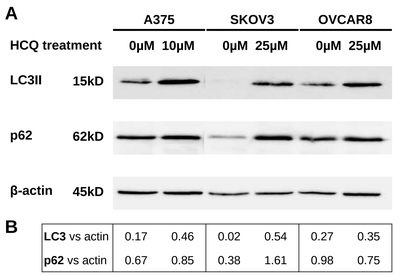


Figure 2. GeoMean Fluorescence Intensity (MFI) levels of PD-L1 across different cell types following HCQ or IFN-γ treatment.
MFI values of : (A) A-375, malignant melanoma cell line; (B) NCI-H2122, non-small cell lung cancer (NSCLC) cell line; (C) U-87 MG, glioblastoma cell line.
Data are presented as mean ± SD (error bars) from three independent experiments. *, p < 0.05; **, p < 0.01; ***p < 0.001; ****p < 0.0001; ns, not significant.

Western blot results indicated that levels of LC3II and p62 increased significantly after HCQ treatment, suggesting that the autophagy process was inhibited (Figure 3). HCQ pre-treatment can significantly elevate the killing efficiency of hmTCRT cells targeting NY-ESO-1 positive A-375 cells (Figure 4). Meanwhile 15μM, 20μM HCQ treatment didn't affect TIL proliferation, which were the same concentrations as treating tumor cells from different patients (Figure 5).



B

LC3 vs actin	0.17	0.46	0.02	0.54	0.27	0.35
p62 vs actin	0.67	0.85	0.38	1.61	0.98	0.75

Figure 3. Western blot analysis of LC3II, p62, and β-actin expression in three human cell lines treated with HCQ.
(A) Representative immunoblots showing protein expression in: Lane 1-2: A-375; Lane 3-4: SKOV3; Lane 5-6: OVCAR-8 with treatments: vehicle control and HCQ. (B) Densitometric quantification normalized to β-actin. Molecular weight markers (kDa) are indicated.

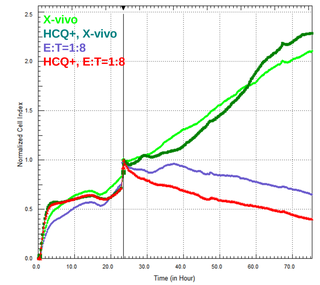


Figure 4. Real-time cytotoxic activity profiling of hmTCRT cells against NY-ESO-1⁺ A-375 melanoma cells under HCQ pretreatment. Dynamic cell index curves monitored by xCELLigence RTCA system (Agilent) over 72 hr with 15-min interval recording. **Light green:** target cells untreated in X-VIVO™ medium(vehicle control); **dark green:** target cells pre-treated with 10 μM HCQ for 48h in X-VIVO™ medium (effector-free control); **purple:** hmTCRT cells co-cultured with target cells at 1:8 E:T ratio; **red:** hmTCRT cells cocultured with target cells pre-treated with 10μM HCQ for 48 hrs prior to 1:8 E:T co-culture.

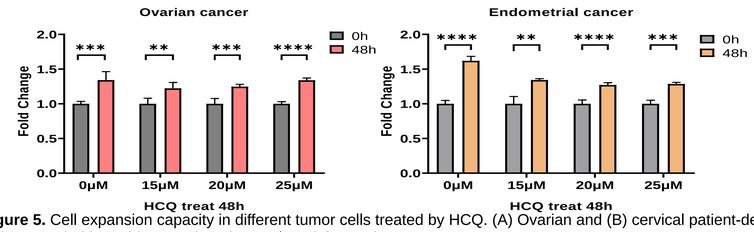


Figure 5. Cell expansion capacity in different tumor cells treated by HCQ. (A) Ovarian and (B) cervical patient-derived cells expanded in rapid expansion phase （REP) for 12 days.

Conclusion:

Our studies indicated that HCQ can significantly increase the tumor killing efficiency via elevating the membrane MHC-I protein levels of tumor cells while it doesn't affect TILs' propagation. In addition, its effect on surface PD-L1 is not significant, which is more advantageous than IFN-γ. These results can provide preclinical evidences for HCQ pretreatment before the adoptive cell therapy of TILs in our clinical trials (NCT06375187).

Abbreviation
HCQ, hydroxychloroquine; MHC-I, major histocompatibility complex class I; NSCLC, non-small cell lung cancer; PD-L1, programmed cell-death ligand 1; RTCA, real-time cell analysis ; TILs, tumor infiltrating lymphocytes.

Disclosures
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