Tumor vulnerability engineering via intracellular IL-24 delivery enhances CAR-T cell cytotoxicity and therapeutic sensitivity

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Abstract

Introduction : Resistance to cancer therapy remains a major challenge across cancers. Here, we identify an intracellular activity of IL-24 that can be therapeutically leveraged. Higher IL-24 level is associated with improved prognosis in BREAST CANCER. We developed a strategy of tumor vulnerability engineering to reprogram tumor cell states and enhance responsiveness to cytotoxic therapies, with particular relevance to military women's health, where breast cancer risk has been reported to be up to 40% higher than in civilians. **Materials and Methods:** Despite its known tumor-suppressive properties, the translational potential of IL-24 has been limited by unclear mechanisms of action and ineffective delivery strategies. We investigated the intracellular function of IL-24 using full-length and signal peptide-deleted variant (IL-24dSP) that bypasses ER-mediated secretion. Intracellular localization and activity were assessed across multiple cancer types, along with its interaction network. A cell-penetrating peptide-fused construct (CPP-IL-24dSP) was engineered to enable intracellular delivery. Functional assays assessed tumor suppression, cytotoxic stress sensitivity (including cisplatin), and susceptibility to CAR-T killing. Extracellular ligand effects were controlled for. **Results:** We found IL-24 engages stress-associated programs converging on PIK3CA- and ATM-centered signaling networks. IL-24dSP, bypassing ER-mediated secretion, shows enhanced intracellular accumulation with prominent cytoplasmic and nuclear foci and confers suppressive activity exceeding that of full-length IL-24, selectively inhibiting malignant phenotypes while sparing normal-like cells. Purified CPP-IL-24dSP enables efficient intracellular delivery and induces potent tumor suppression, increasing sensitivity to cisplatin and CAR-T killing. In contrast, extracellular IL-24dSP lacking CPP, functioning solely as a ligand, fails to produce these effects. Ongoing studies focus on developing CAR-T cells for activation-dependent co-delivery of CPP-IL-24dSP to precisely induce tumor vulnerability, using ex vivo co-culture systems and in vivo syngeneic models, including in combination with cisplatin. **Conclusions:** Intracellular delivery of engineered IL-24dSp represents a tumor vulnerability engineering strategy that sensitizes tumor cells to cytotoxic therapies, enabling CAR-T-based, CPP-IL-24dSp co-delivery and Chemo-IO combination approaches to overcome therapeutic resistance and improve efficacy.

Results

1. IL-24 level is associated with better survival in BRCA, and the **rare** tumor cell-intrinsic IL-24$^+$ cells underlies a tumor-suppressive signature.

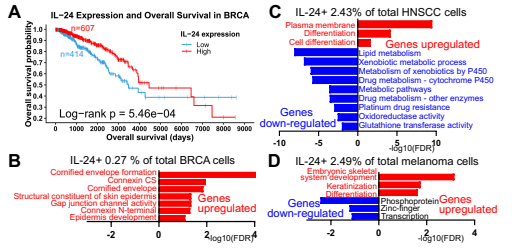


Figure 1. IL-24-expressing tumor cells represent a rare population but tend toward differentiation and reduced malignancy in BRCA, HNSCC and melanoma. A, Kaplan–Meier analysis of overall survival in the TCGA breast cancer cohort stratified by IL-24 expression using the Human Protein Atlas cutoff of 0.35 TPM; **B-D,** IL-24-expressing tumor cells from BRCA, HNSCC and melanoma datasets (GSE176078, GSE103322 and GSE115978) were identified and re-analyzed for differentially expressed genes (DEGs), followed by pathway enrichment analysis using DAVID. Red: Pathways enriched for upregulated genes in IL-24$^+$ tumor cells. Blue: Pathways enriched for downregulated genes in IL-24$^+$ tumor cells.

Results

2. IL-24 non-secreted variant IL-24dSP exhibiting enhanced foci-like accumulation in both the cytoplasm and nucleus, and stronger suppressive effects in cancers cells.

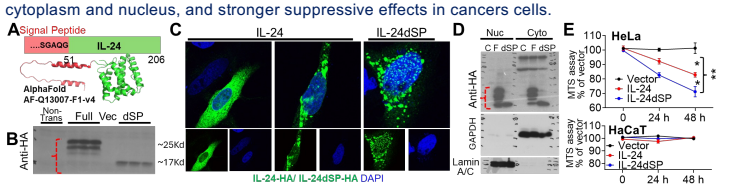


Figure 2. The distribution pattern and suppressive effects of IL-24 and its non-secreted variant IL-24dSP in HeLa cells. A. Human IL-24 sequence overview. The signal peptide is highlighted in red in both the structural domain model and the 3D model of IL-24. **B.** Expression of IL-24 and the non-secreted variant in HeLa cells transiently transfected with either C-terminal HA-tagged IL-24 or IL-24dSP (in the pMXs-EF1-puro vector), analyzed by Western blot. Non-trans: Non-transfected HeLa cells; Vec, HeLa cells transfected with vector; Full: Full-length IL-24; dSP: IL-24 lacking the signal peptide (non-secreted version); **C.** Confocal images of HA-tagged IL-24 or IL-24dSP in HeLa cells transiently transfected with either C-terminal HA-tagged IL-24 or IL-24dSP. Images show representative anti-HA (IL-24) immunostaining in transfected cells. **D.** HeLa cells transiently transfected with either C-terminal HA-tagged IL-24 or IL-24dSP were subjected to cell fractionation followed by western blot analysis. Nuc, Nuclear; Cyto, cytoplasm. **E.** HeLa and HaCaT cells were transiently transfected with either vector control, IL-24 or IL-24dSP (in the pMXs-EF1-puro vector), and subjected to MTS assays at 24 and 48 hours post-transfection. IL-24- or IL-24dSP-expressing groups were compared to the vector control group .n=3 technical repeat. ± SEM. ** p < 0.01; * p < 0.05; by t-test.

3. IL-24 physically engages unique networks converging on PIK3CA- and ATM-centered signaling programs.

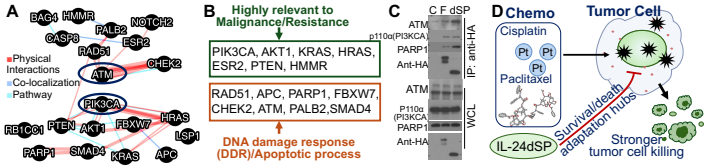


Figure 3. Functional Network Analysis of IL-24-Associated proteins based on deposited datasets. A. List of IL-24-interacting proteins associated with cell survival and cancer. Data were re-processed from a previously published yeast two-hybrid screen. Internal connectivity was analyzed using GeneMANIA, incorporating physical interactions, colocalization, and shared pathway associations. Notably, the network highlights a strong connection to PIK3CA and PI3K-like kinase ATM. **B.** Categorization of IL-24-interacting proteins reveals two major biological processes potentially modulated by IL-24: malignancy/resistance and DNA damage response (DDR)/apoptosis. **C**. Doxycycline-induced(1 ug/mL) HeLa cells expressing HA-tagged human full-length IL-24 or non-secreted variant (IL-24dSP) were subjected to anti-HA IP and Western blot. **D.** Preliminary evidence that IL-24/IL-24dSP sensitizes cancer cells to chemotherapy-mediated killing across multiple cancer types

4. Cell-penetrating IL-24 (CPP-IL-24dSP) induces broad tumor-suppressive/apoptotic effects.

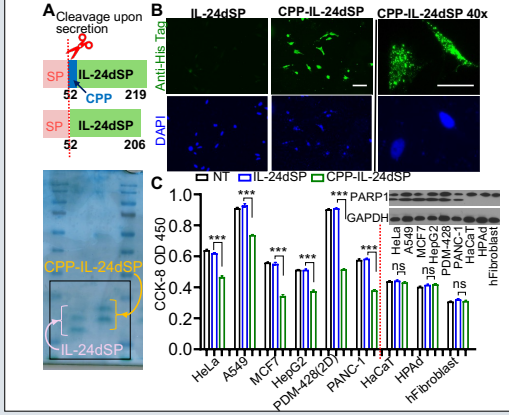


Figure 4. Cell-penetrating IL-24 enables intracellular delivery and induces broad tumor-suppressive effects. A-B. Recombinant CPP-IL-24dSP or IL-24dSP lacking the CPP(**A**) (produced in HaCaT cells and purified from culture media via a C-terminal His tag; CPP-IL-24dSP were tested for cell delivery into HeLa cells for 24 h (1 μg/ml), followed by anti-His staining (**B**). Scale bar, 100 μm and 50 μm. **C.** Indicated cells were treated with CPP-IL-24dSP or IL-24dSP for 48 hours, followed by CCK-8 viability assay (incubation for 3 h, n=5 or western-blot. All bar graphs, ± SEM. *** p < 0.001, ** p < 0.001 , * p < 0.05; ns, not significant; by t-test.

Results

5. Cell-penetrating IL-24 (CPP-IL-24dSP) significantly augments cisplatin-mediated tumor cell killing (data not shown) and CAR-T cell-mediated cytotoxicity.

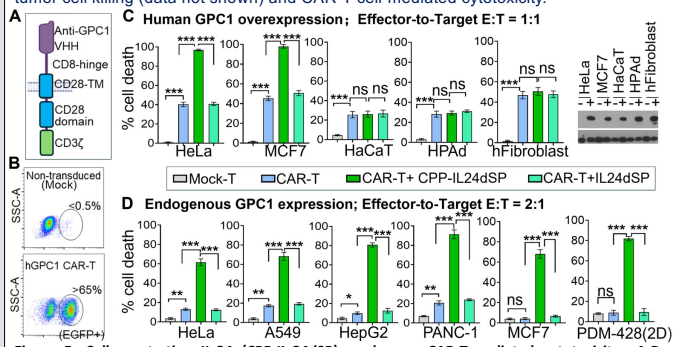
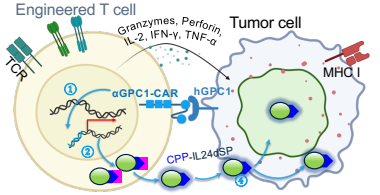


Figure 5. Cell-penetrating IL-24 (CPP-IL-24dSP) enhances CAR-T-mediated cytotoxicity. .A-B. Schematic of the anti-hGPC1 CAR construct with an EGFP reporter (**A**) and flow cytometric validation of expanded T cells derived from PBMCs following transduction with the indicated CAR construct (**B**), achieving representative >65% transduction efficiency. Mock, non-transduced. **C.** Cell killing assays were performed by co-culturing indicated cells ectopically expressing GPC1, with anti-GPC1 CAR-T cells at an effector-to-target ratio of 1:1. Western blot confirmed leniviral overexpression of Flag-tagged GPC1 in indicated cells. **D.** A representative panel of cancer cells with known basal GPC1 expression was subjected to tumor cell killing assays using anti-GPC1 CAR-T cells at an effector-to-target ratio of 2:1. Cytotoxicity for (**C-D**) was measured by LDH releasing assay following 16-hour co-culture of CAR-T cells w/ or w/o purified CPP-IL-24dSP or IL-24dSP lacking the CPP (1 μg/mL). Mock-T (Non-transduced T cells) were included as controls to assess basal allogeneic cytotoxicity. n=3. All bar graphs, ± SEM. *** p < 0.001, ** p < 0.001 , * p < 0.05; ns, not significant; by t-test.

Conclusions and Ongoing Studies

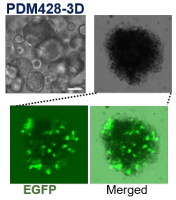
Intracellular delivery of engineered IL-24dSp represents a tumor vulnerability engineering strategy that sensitizes tumor cells to cytotoxic therapies and is being actively advanced through CAR-T-based, CPP-IL-24dSp co-delivery and potential Chemo-IO combination approaches to overcome therapeutic resistance and improve efficacy in breast cancer and other malignancies.

A delivery platform

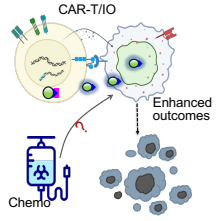


A co-culture testing system

Organoids + αGPC1-CAR-T



A combination roadmap



Ongoing: 1. Establishing activation-coupled CPP-IL-24dSP co-delivery in human CAR-T systems and in mice model.

2. Defining the molecular mechanisms by which intracellular IL-24 rewires interconnected ATM, PI3KCA- centered survival/death networks to sensitize tumors, using integrative transcriptomic, proteomic, and functional analyses to identify the key regulatory pathways and hubs.

3. Evaluating minimal functional domains/ bioactive peptides and optimizing delivery strategy