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Targeting lineage plasticity overcomes chemoresistance

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In this issue of *Cancer Cell*, Wang et al. reveal that chemoresistant muscle-invasive bladder cancer is associated with partial squamous differentiation. Targeting of Cathepsin H overcomes this chemotherapy-induced semi-squamatization and promotes terminal squamous differentiation and tumor suppression.

Chemoresistance is one of the largest barriers standing in the way of a cure for cancer. Many cancer patients can respond to chemotherapy once and even achieve complete disappearance of tumors. Unfortunately, however, the majority of chemotherapy patients ultimately experience cancer recurrence, eventually resulting in death. Thus, overcoming chemoresistance is a key to curing cancer. To date, several mechanisms have been implicated in chemoresistance (Vasan et al., 2019). Under discussion for some time has been the role of cancer stem cells (CSCs), in which a small number of immature cells (also called "cancer-regenerating cells") that can regenerate entire cancer populations survive chemotherapy due to their slow cell cycling and/or intrinsic detoxicating machineries, and these cells contribute to relapse (Valent et al., 2012). However, this does not seem to explain why tumors become increasingly resistant to repeated cycles of chemotherapy. Lineage plasticity is another common mechanism commonly used to explain chemoresistance. With lineage plasticity, chemoresistance can be achieved by switching the cell state (Le Magnen et al., 2018; Quintanal-Villalonga et al., 2020) via epithelial-mesenchymal transition (Dongre and Weinberg, 2019).

In this issue of *Cancer Cell*, Wang and colleagues reveal a unique mechanism of chemoresistance that is explained by lineage plasticity in muscle-invasive bladder cancer (MIBC) (Wang et al., 2022). MIBC is a common subtype of bladder cancer with a high mortality rate, and it is characterized by a basal-cell-like gene expression profile (Tran et al., 2021). Although cisplatin-based chemotherapy remains the mainstay of therapeutics in unresectable and metastatic

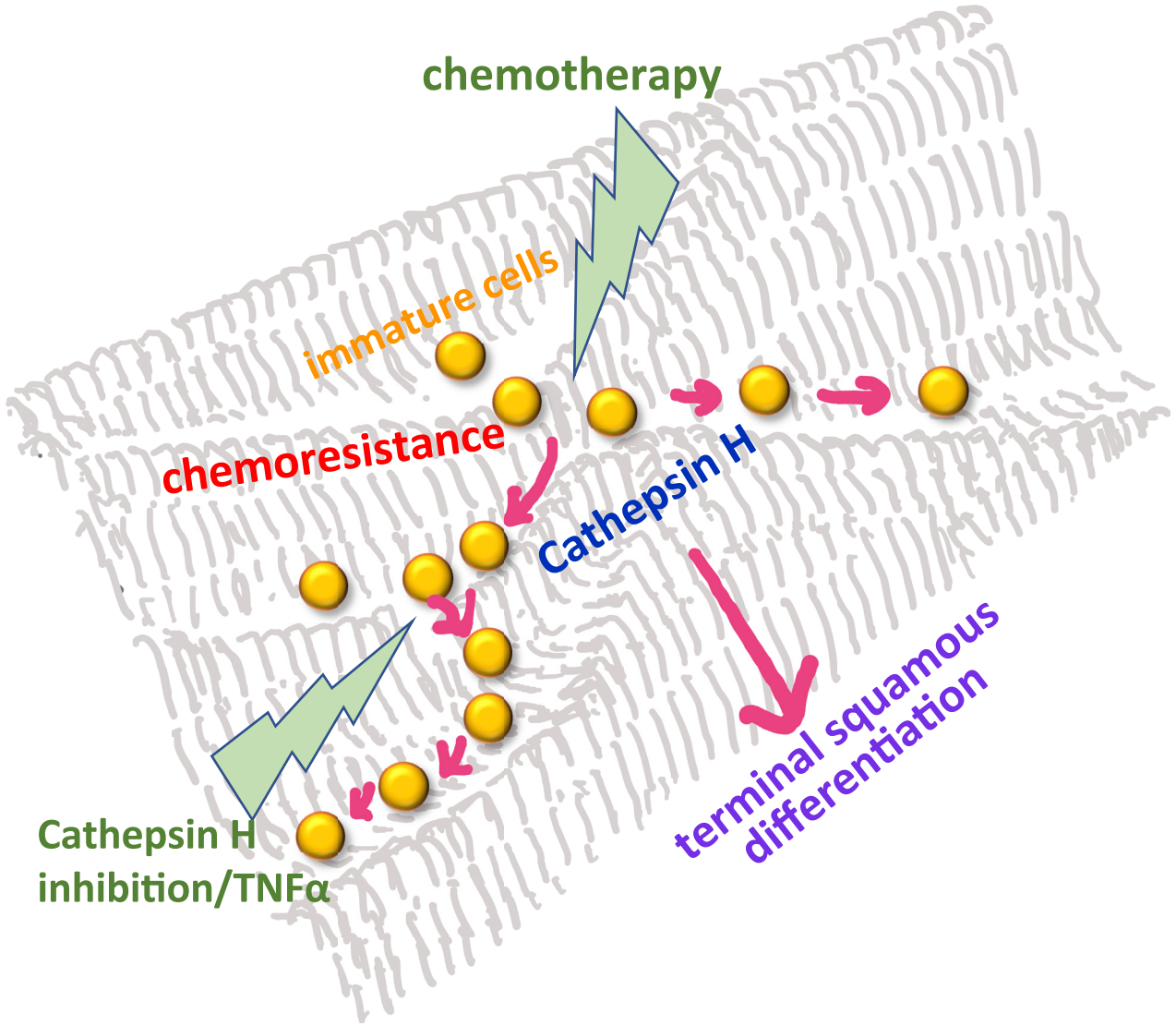

Figure 1. Linage plasticity dictates chemoresistance and squamous differentiation
Chemotherapy kills tumor cells and initiates squamatization in immature tumor cells. However, this differentiation is blocked by Cathepsin H, leading to chemoresistant semi-squamatized cells. Inhibition of Cathepsin H promotes terminal squamous differentiation, leading to TNFα-signaling-dependent pyroptosis and tumor regression

MIBC tumors, most tumors ultimately become chemoresistant during repeated chemotherapy cycles. To investigate the mechanism of the progressive chemoresistance in MIBC, Wang et al. established a therapeutic model of MIBC by engineering bladder-derived mouse organoids with disrupted *Tp53* and *Pten* and others with *Myc* overexpression. When orthotopically transplanted into the mouse bladder, the engineered organoids developed undifferentiated tumors that mimicked human MIBC in terms of molecular and histological features. In particular, mouse MIBC tumors, which initially responded to cisplatin + gemcitabine, soon relapsed, and their tumors were highly chemoresistant and positive for squamous cell markers such as EPCAM, CK5, CK14, and p40, which were not expressed in original chemosensitive tumors.

Single-cell RNA sequencing (scRNA-seq) of these chemoresistant tumors revealed two discrete populations, T1 and T2. Although chemosensitive tumors contained a minor fraction of T2 cells, T2 cells were dominant in chemoresistant tumors and were responsible for chemoresistance as revealed by secondary transplantation of these cells. Pseudotime analysis using dynamically expressed genes revealed a trajectory from T1 to T2 in chemosensitive tumors, followed by T2 cells in chemoresistant tumors. In this trajectory, gene sets that were enriched in the pathways related to keratinocyte differentiation and squamous signature were gradually upregulated, whereas most of the stem-cell-related gene sets were negatively enriched. This suggests that transition from T1 to T2 in pseudotime analysis represents a type of squamatization process, and this was supported by histology that revealed obvious squamous differentiation. *In vivo* lineage-tracing using DNA barcodes before and after chemotherapy confirmed that the expanded T2 population in

chemoresistant tumors resulted not from a positive selection of a small number of clones, but rather from squamatized differentiation of the highly polyclonal bulk tumor population. Lineage tracking using a mCherry reporter driven by the *EPCAM* promoter showed a gradual increase in mCherry intensity but not in the number of mCherry$^+$ cells, and this finding also supports linage plasticity as the mechanism of chemoresistance. A similar mechanism of chemoresistance upon repeated cycles of chemotherapy was demonstrated using human MIBC *in vitro* or in patient-derived xenograft (PDX) models, and this further supports a role for lineage switching in chemoresistance.

Wang et al. next investigated the molecular basis of this linage plasticity and chemoresistance during chemotherapy by using multi-omics analysis, including assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) and proteomics. In agreement with the gene expression analysis, ATAC-seq revealed an enhanced accessibility of genes related to squamatization and keratinocyte differentiation pathways in chemoresistant tumors. When the authors combined the results with the proteomics analysis, they identified 49 genes that showed enhanced accessibility and gene expression across all platforms. From this list, the authors focused on Cathepsin H (CTSH), a lysosomal cysteine protease. The Cathepsin family of proteins has been implicated in therapy resistance in various human cancers (Olson and Joyce, 2015; Zheng et al., 2004). A gradual increase in *CTSH* expression was shown along the chemoresistance trajectory in both human and mouse MIBC, and a stepwise increase was seen with multiple rounds of chemotherapy. These results suggest that CTSH might be responsible for the chemoresistant phenotype of MIBC. In fact, CRISPR-Cas9-mediated disruption of *Ctsh* caused growth suppression of chemoresistant organoids with minimum effects on chemosensitive tumors, both *in vitro* and *in vivo*. Conversely, overexpression of *Ctsh* in chemosensitive organoids significantly accelerated relapse and tumor growth with higher expression of squamatization-related genes, such as *keratin* (*Krt*)*5* and *Krt14*. In line with this, chemoresistant organoids were more sensitive to a Cathepsin inhibitor, E64, compared to chemosensitive organoids, and E64 treatment significantly suppressed tumor growth. Of particular interest is the finding that E64-treated tumors exhibited large areas that showed keratin pearls and concentric layers of keratin deposition with surrounding differentiated keratinocyte-like cells, suggesting that the E64-induced growth suppression was accompanied by squamous differentiation. Importantly, gene expression, proteomics, and scRNA-seq data showed that E64-treated chemoresistant tumors had more prominent upregulation of squamous differentiation-related genes and accessible chromatin than untreated tumors had, suggesting that squamous differentiation is an epigenetically regulated process. E64-induced growth suppression and squamatization was also demonstrated in a PDX model of human MIBC. Of interest, the authors demonstrated that E64-treated chemoresistant tumors showed elevated levels of TNFα. Treatment with recombinant TNFα reduced the survival of chemoresistant organoids with elevated expression of squamous-differentiation-related genes in a dose-dependent manner. In contrast, TNFα neutralizing antibody or CRISPR-Cas9-mediated disruption of *Tnfr1* rescued the suppression and squamous differentiation of chemoresistant tumors upon E64 treatment, and this finding highlights a critical role of TNFα signaling in cell differentiation and growth suppression upon CTSH inhibition.

To summarize, when exposed to chemotherapy, immature MIBC cells initiate a differentiation program toward squamatized cells. Unfortunately, due to CTSH upregulation, this chemotherapy-induced squamatization process is only partially completed—hence the term "semi-squamatization" (Figure 1). This process represents another example in which lineage plasticity plays a major role in chemoresistance. Recent research has shown several examples in which chemoresistance was induced by lineage plasticity (Dongre and Weinberg, 2019; Zou et al., 2017). However, what makes Wang et al.'s study unique is the finding that the linage plasticity that induces the chemoresistance could also be exploited to develop novel differentiation therapy for MIBC. The authors showed that the blocked differentiation can be released by inhibiting CTSH activity via CRISPR-Cas9-mediated gene knockdown or enzymatic inhibition using E64, which in turn enable semi-squamatized tumor cells to terminally differentiate and achieve tumor regression and TNFα-signaling-dependent pyroptosis. Although the role of Cathepsin inhibition in the treatment of advanced MIBC still awaits proof-of-concept clinical trials, these findings clearly show that lineage plasticity underlying chemoresistance might be exploited for treatment, and this research sheds light on a new possibility of differentiation therapy for many epithelial cancers.

DECLARATION OF INTERESTS

The author declares no competing interests.

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