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NEWS

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METASTATIC RECURRENCE IN COLORECTAL CANCER ARISES FROM RESIDUAL EMP1+ CELLS

In localized colorectal cancer (CRC), surgical resection of the primary tumor effectively cures most patients. However, ~30%-40% of them will eventually develop metastases. Therefore understanding biological and molecular patterns of the early stages of metastases, as well as identifying novel targeted therapies is needed to improve patient outcomes. Cañellas-Socias et al.[1] recently published an inspiring article in *Nature* that focuses on the identification and characterization of the residual tumor cells responsible for CRC relapse. First, in this study, from a large transcriptomic cohort of patients with CRC, they were able to determine a set of genes associated with poor prognosis; 99 of these recurrence-associated genes were found to be upregulated in the tumor epithelial cells, named as epithelial-specific high-risk gene set (EpiHR). Characterizing the cell populations enriched in this gene signature, they highlighted the LGR5+ stem cell-like cells, whose relationship with metastasis had been previously described. In addition, they pointed to a different population called high-relapse cells (HRCs), which not only expressed the EpiHR signature, but also genes enriched in cell migration or cell-to-cell adhesion functions.

Based on these findings, they carried out an assay to characterize this population, injecting mouse tumor organoids and subsequently carrying out resection of the primary tumor once the metastasis had been developed. Comparing human and mouse single-cell RNA-sequencing data they identified *Emp1*, a gene that was expressed at high levels in HRCs, and demonstrated a high level of overlap with the expression of the EpiHR gene set in both human and mouse CRCs. After marking the locus of this gene with a fluorescence reporter (TOM) by means of a clustered regularly interspaced short palindromic repeats—CRISPR-associated protein 9 (CRISPR—Cas9) strategy, the high-intensity EMP1-TOM+ cells were found at the boundaries of the tumor in contact with the fibroblast population and invading the muscle layer. By contrast, the LGR5+ cell population was found in the central part of the tumor. Together with bioinformatics predictions and single-cell RNA results, which showed that micrometastases were enriched by undifferentiated HRCs, this evidence suggested that these cells were responsible for the migration and dissemination of the tumor to distant organs and, hence, those responsible for the onset of metastasis.

The importance of this cell population in the generation of metastasis raised the question of whether its elimination could stop this process. Thus they performed a cell ablation on the high EMP1-TOM+ cells. When this ablation took place before the primary tumor resection, it had no effect on the growth of the primary tumor itself, but it had a dramatic effect on metastatic capacity, as most mice did not show metastatic recurrence. However, when the ablation was carried out 1 week after primary CRC resection, the metastatic process was not modified and all mice underwent metastatic relapses. By contrast, killing LGR5+ cells before surgical removal of the primary tumor had no effect in preventing distant relapses, while if this procedure was carried out once the metastasis has already been implanted, the metastatic outgrowth was affected. To test a reproducible treatment strategy in humans and provided that when micrometastases are forming there is a strong inflammatory response and a high expression of programmed death-ligand 1, they treated mice with neoadjuvant checkpoint inhibitors, observing the same response as with cell ablation. The fact that immunotherapy is effective in eliminating minimal residual disease and preventing subsequent metastatic relapse could be of paramount importance.

In conclusion, the identification of HRCs as being responsible for the metastatic onset is a very important finding that opens some potential clinical applications, with special emphasis on the treatment of patients prior to surgery. Until now, adjuvant chemotherapy was trying to tackle an already established micrometastatic disease. However, this study has shown that if the window of vulnerability of the process of metastasis development is taken advantage of, prior to the formation of a mature tumor microenvironment, treatment with neoadjuvant immunotherapy could successfully prevent relapses. The addition of this or similar strategies in future clinical trials is needed to further prove the value of these findings for patients with localized colon cancer.

AXL AND ERROR-PRONE DNA REPLICATION AS A MECHANISM OF RESISTANCE TO EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) INHIBITORS AND POTENTIAL STRATEGIES TO CIRCUMVENT IT IN EGFR-MUTANT NON-SMALL-CELL LUNG CANCER

Resistance to targeted agents represents the most relevant limitation of the cancer molecular-matched approach. For this reason, understanding the mechanisms of drug-tolerant persister cells is a priority. Epidermal growth factor receptor (EGFR)-mutant non-small-cell lung cancer is a specific entity and one of the most relevant paradigms of precision oncology. During the past few years, three generations of tyrosine kinase inhibitors (TKIs) have been developed to treat patients with EGFR-mutant non-small-cell lung cancer with a consistent improvement in clinical outcomes. However, acquired resistance in previously sensitive patients will eventually occur,

thus limiting its curative potential. Despite a wide variation in the molecular mechanisms responsible for this phenomenon, such as MET amplification and stimulation of epithelial—mesenchymal transition among others, all finally converge in the presence of *de novo* mutagenesis and bypass routes[2] able to develop drug-tolerant persister cells.[3]

In an article recently published in *Cancer Discovery,* a multinational group of investigators lead by Yosef Yarden[4] at the Weizmann Institute of Science in Israel sought to underline novel mechanisms responsible for resistance to EGFR TKIs. The authors investigated whether the treatment of lung cancer with EGFR TKIs causes the selection of a hypermutator phenotype, which is finally responsible for resistance.

To demonstrate so, they treated EGFR-mutant cell lines with erlotinib to evaluate possible mechanisms of resistance. Through RNA analysis they were able to identify GAS6 (growth arrest-specific gene 6) upregulation that was the highest in those cycling persister cells. Consistent with these results, the single-cell RNA-sequencing data obtained in a cohort of patients exposed to osimertinib showed across untreated patients, as well as in patients who experienced progression, low or undetectable GAS6. By contrast, the majority of the residual disease group displayed high GAS6, underlying the relevance of AXL in this process. To further evaluate the mechanisms responsible for the drug-tolerant persister cells profile, the authors studied the effects of AXL on DNA breaks. As expected, exposure of lung cells to EGFR inhibitors increased apoptosis and reduced viability, whereas AXL overexpression enhanced viability and reduced apoptosis. Moreover, TKIs increased DNA breaks while AXL inhibited them. In particular, AXL was found to be able to interact with the DNA damage repair protein RAD18 and DNA polymerases specialized in translesion synthesis inducing neddylation. Moreover, it was found that in response to TKIs, AXL levels were enhanced and activated MYC, which might increase purine mutational bias that might further augment mutagenesis. In the studied models *in vitro* and in *vivo,* when AXL was blocked with specific monoclonal antibodies, it was possible to restore the transition of the drug-tolerant persister cells to resistant phenotype. In line with the AXL-dependent conversion of drug-tolerant persister to resisters cells, a triplet containing a novel anti-AXL antibody inhibited the mutators and could restore sensitivity to TKIs. Although further investigations are needed, these observations may have relevant clinical implications. TKI-treated tumors may present some acquired pharmacologic vulnerabilities and AXL inhibition may help in avoiding progression of EGFR-mutant tumors, opening a new field of active clinical research.

THE TARGET ANTIGEN DETERMINES THE MECHANISM OF ACQUIRED RESISTANCE TO T-CELL-BASED THERAPIES

Immunotherapy is revolutionizing the management of several solid tumors. Current clinical trials are focused on developing immunotherapy combinations and new strategies. In this scenario, bispecific antibodies (TCBs) or chimeric antigen receptors have emerged. They are engineered molecules that include binding site to the T-cell receptor and to a tumor antigen. In fact, several studies underlined that the antigen loss is one of the most frequent mechanisms of resistance to these therapies.

Martinez-Sabadell et al.[5] published in *Cell Reports* a relevant article where they describe different mechanisms of resistance to TCBs depending on the target tumor antigen. The authors used a model of a gastric HER2+ and CEA+ MKN45 cell line and TCBs targeting CEA or HER2. They generated a resistance model treating *in vitro* the gastric cancer CEA+/HER2+ MKN45 cell in coculture either with high-affinity CEA-targeting TCB (CEACAM5-TCB) or with HER2-TCB. As a result, they generated two cell lines, MKN-HER2R, resistant to HER2-TCB, and MKN-CEAR, with lower sensitivity to CEACAM5-TCB.

First, they analyzed protein levels of both CEA and HER2 in acquired resistant cells. They showed that MKN-CEAR presented lower CEA protein expression; by contrast, MKN-HER2R maintained levels of HER2. Interestingly, they assessed lymphocyte activation and functionality. The results reveled that the activation of lymphocytes by MKN45 and the MKN-HER2R was very similar; however, in MKN-CEAR this was significantly reduced. This evidence suggests that lymphocytes cannot be properly activated due to the decrease in the level of the antigen. In addition, they demonstrated that this regulation was at the transcriptional level. These results were confirmed by generating different cell lines and patient-derived xenograft models with acquired resistance. Second, they described an alternative mechanism of acquired resistance independent of the antigen presentation in the HER2-TCB-resistant model. They explored interferon-γ signaling, a pathway that is essential for the response to the redirection of T lymphocytes.[6] The authors confirmed a downregulation of interferon-γ signaling in acquired resistance cells maintaining antigen levels.

Overall, this work has practical implications when designing combinatorial strategies to increase the efficacy of cancer immunotherapies. The selection of a particular antigen may have an impact on the type of mechanism of acquired resistance that emerges in refractory patients to T-cell-redirected therapies.

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