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PHARMACOLOGICAL ACTIVATION OF P53 TRIGGERS VIRAL MIMICRY RESPONSE, CONSEQUENTLY ABOLISHING TUMOR IMMUNE EVASION AND PROMOTING ANTITUMOR IMMUNITY

Reinstatement of the major tumor suppressor p53 demonstrated remarkable cancer inhibition, providing strong support for p53 reactivation as an anticancer strategy. P53, via direct binding and/or cooperation with epigenetic mechanisms, exerts part of its activity inhibiting the expression of repetitive elements, in particular in endogenous retrovirus (ERV)-derived long terminal repeat sequences.[1] The genetic or pharmacologic induction of ERVs expression is known to trigger a viral mimicry response leading to an alteration in the local tumor microenvironment. This change was related to an interferon (IFN)-type response that could boost response to checkpoint inhibitors.[2] Despite several shreds of evidence, however, the effect of pharmacologically activated p53 on the anticancer immune response is still not clear. During the past years, murine double minute protein (MDM2) inhibitors able to restore the function of wild-type p53 have been developed. These drugs act by targeting their major negative regulators MDM2 or/and MDMX and are under evaluation for cancer patients.

In a relevant article published in *Cancer Discovery* by Zhou et al.,[3] the authors demonstrated that pharmacological activation of p53 induces the expression of ERVs and generation of double-stranded (ds) RNA which caused intracellular dsRNA stress leading to type 1 and type 3 IFN responses and induction of amyloid beta precursor protein or APP genes. Interestingly, the authors found that p53 activation promotes the recruitment of immune cells to tumors *in vivo* in mouse models and sensitizes refractory tumors to programmed cell death protein 1 (PD-1) blockade. In their experiments, pharmacologically activated p53 induces the transcription of a subset of ERVs in human and mouse cells, which leads to the generation of dsRNA and dsRNA stress that triggers viral defense responses. They show that p53 accumulation through the novel compounds causing MDM2 inhibition is associated with increased occupancy of p53 on ERV promoters. Moreover, they demonstrated the p53-mediated inhibition of two major repressors of ERVs, histone demethylase LSD1 and DNA methyltransferase DNMT1. This phenomenon contributes to the de-repression of ERVs, however, the contribution of different repetitive sequences to the shaping of the immune microenvironment remains to be clarified.

Across melanoma patients treated with the experimental MDM2 inhibitor ALRN-6924, the analysis of pre- and post-treatment tumor biopsy samples revealed the induction of viral mimicry response genes, as well as immune function signatures suggesting infiltration of cytotoxic CD8+ T cells. Several previous studies have described the effect of pharmacological activation of p53 on antitumor immunity, including IFN induction, the polarization of macrophages, induction of programmed death-ligand 1 (PD-L1), and potentiation of immune checkpoint inhibitors. The results presented in this article are very encouraging and suggest the potential role of combining MDM2 inhibitors with checkpoint inhibitors and wild-type p53 status might serve as one important biomarker. As conclusions, the data presented across cancer cell lines, tumor-bearing mouse models, and melanoma patients suggest that pharmacological p53 reactivation triggers the ERV-dsRNA-IFN pathway within tumor cells, thereby altering the tumor microenvironment evoking tumor immune surveillance.

ARTIFICIAL INTELLIGENCE MAY PREDICT PRIMARY ORIGIN OF TUMORS FROM PATHOLOGY SLIDES

Despite enormous advances in tumor characterization with the incorporation of immunohistochemistry and molecular biology, there are still some dramatic cases that remain without an accurate diagnosis. As current cancer treatments largely depend on tumor origin, patients diagnosed with cancer of unknown primary are not able to receive the best treatment option and have a poor prognosis. Recent approaches to improve identification of tumor origin include genomic and transcriptomic analyses.[4,5] Routine pathology slides are also a vast source of information that the human eye can possibly only partially identify and interpret. Several deep learning applications in pathology have been reported that are able to predict molecular alterations and clinical outcome.[6,7]

In an exciting work by Lu and colleagues[8] published in *Nature*, an artificial intelligence-based system successfully predicts the origin of unknown primary tumors from pathology slides. The authors present an algorithm called TOAD (Tumour Origin Assessment via Deep Learning) that uses hematoxylin–eosin whole-slide images. The system was developed with >32 000 digitized slides with confirmed diagnosis from 18 common tumor origins, including both publicly available and in-house slides. The algorithm was trained on >22 000 slides combining transfer learning and weakly supervised multitask learning. Regions in the slide that are of high diagnostic relevance are identified using attention-based learning. Importantly, the attention heat map can be visualized for each slide for human validation, as is nicely shown on the TOAD interactive demo website.

The model was first tested on 6499 digitized slides from tumors with known primary origins, achieving an overall

accuracy of 83.4%, which increased to 95.5% when evaluated using the top-3 differential diagnosis accuracy. The TOAD algorithm was also able to predict whether the tumor specimen was primary or metastatic. Ablation experiments showed an increased performance when the algorithm was trained, including cases of primary tumors. Interestingly, the model also benefited from incorporating the sex of the patient into the prediction, as one would expect from a pathologist looking at the slide. In contrast, adding the tissue sampling or biopsy site decreased the model accuracy. As suggested by the authors, this probably provided a direct shortcut to the ground truth label and discouraged from learning from the morphology of primary tumors, perhaps reminding why pathologists prefer to start viewing the slide blind.

The system was next evaluated on a cohort of 682 external samples from >200 centers, achieving an accuracy of 79.9% and a top-3 accuracy of 93.4%. Validation of digital pathology models in external slides is crucial to include different staining protocols and ensure future usefulness. A major challenge of the model is that there is no certain diagnosis to compare to when a case is catalogued as cancer of unknown primary. The authors analyzed increasingly complex cases with known primary and achieved in the most difficult cases a top-1 accuracy of 75.7% and a top-3 accuracy of 92.0%, using only routine slides and the sex of the patient as input. Finally, the model was tested on a set of 317 challenging unknown primary cancer cases, which had been assigned a primary differential diagnosis based on an extensive multidisciplinary work-up. The prediction was concordant with the primary differential in 60.6% of the patients, reaching 82.0% when considering the top-3 predictions. The possibility of narrowing down the options underscores the potential of artificial intelligence to contribute to an efficient diagnosis, reducing patient invasiveness and ancillary tests.

As shown by this and other recent stimulating research, artificial intelligence can provide deep analysis of histological slides and complement histopathological analysis. Digital pathology has proven its viability even more in the COVID era, and algorithms will probably reduce diagnosis time and detect clinically relevant features. Nevertheless, human evaluation and interaction with the systems will undoubtedly remain essential. Even though implementation of artificial intelligence into clinical practice is still challenging, we may be facing another pillar of an integrated personalized cancer diagnosis.

CO-OCCURRING GAIN-OF-FUNCTION MUTATIONS IN HER2 AND HER3 MODULATE HER2/HER3 ACTIVATION, ONCOGENESIS, AND HER2 INHIBITOR SENSITIVITY

Human epidermal growth factor receptor 2 (HER2) amplification or overexpression is detected in 20%-30% of patients with breast cancer and is associated with a poor prognosis. Overexpression of this protein is an important predictive biomarker for identifying patients who may benefit from HER2-targeted therapy. The mechanisms of HER2 activation include not only the overexpression of the protein, however, but also somatic mutations in *HER2*, leading to activation of the gene.

In breast cancer, these mutations are more common in invasive lobular types and typically occur in the absence of *HER2* amplification. *HER2* mutations have been reported in ~5% of breast cancers and have been associated with poor prognosis and are related to hormonal therapy resistance. Neratinib is an irreversible pan-HER tyrosine kinase inhibitor that has shown activity in HER2-mutant cancers.[9]

Hanker et al. published[10] an interesting article in *Cancer Cell* showing how mutant HER3 receptor cooperates with mutant HER2 to promote tumor growth via enhanced HER2 and phosphoinositide-3-kinase (PI3K) activation. Mutations in HER2 and HER3 showed a significant tendency to co-occur in breast cancers. Interestingly, they showed that HER3 mutation did not co-occur when HER2 was both mutated and amplified. Moreover, in HER2-mutant tumors, HER3 and PIK3CA were mutually exclusive, suggesting that HER3 and PIK3CA mutations are functionally redundant.

The authors demonstrated that HER3[11] mutations increased dimerization affinity to HER2 and enhanced kinase domain (KD). In fact, concurrent HER2/HER3 mutants enhanced ligand-independent PI3K activity, which was associated with increased growth and invasion. Moreover, the HER2- and HER3-targeting antibodies did not disrupt the signaling of $HER3^{E928G}$ with HER2. This result suggested that $HER3^{E928G}$ may enable the intracellular association of HER2 and HER3 KD mutants, even when the extracellular domain interaction is disrupted by antibodies. To complete the analyses, they demonstrated that cancers bearing $HER3^{E928G}$ mutations had reduced sensitivity to neratinib.

Finally, due to the fact that HER2/HER3 co-mutations hyperactivated the PI3K/AKT pathway, they assessed the role of PI3K inhibitors. As they expected, the combination of PI3Kα increased the sensitivity of tumors with $HER2^{mut}$/$HER3^{E928G}$ to HER2 tyrosine kinase inhibitors. Overall, this is a motivating article on HER2 and HER3 concomitant mutations, genes that encode members of the same signaling complex. Future studies should address the role of concomitant HER2/HER3 mutations and the potential benefit of PI3Kα inhibitors.

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