and presents an interesting target to develop therapeutics.

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Forum

Engineering Tumor Hypersusceptibility to Checkpoint Immunotherapy

Ariel Fernández[1,2,3,*]

The immune checkpoint blocker pembrolizumab (Keytruda) has proven successful in treating solid tumors harboring a DNA mismatch repair (MMR) deficiency. We show that it is possible to generate a drug-promoted phenotype mimicking the MMR deficiency in solid tumors, and thereby to engineer a generic hypersusceptibility to Keytruda through drug-induced metabolic stress on DNA synthesis. The potential of such drug–Keytruda combinations as universal treatments for solid tumors deserves clinical evaluation.

Checkpoint Blocker Success in the Mismatch Repair Deficiency Phenotype

Merck's drug Keytruda (pembrolizumab) unleashes antitumor immunity by suppressing a negative signal or checkpoint of the adaptive immune response[i]. On the other hand, Vogelstein *et al*. showed that cancer gene mutations can be presented in surface protein fragments by the major histocompatibility complex recognized by T cells, and conjectured that hypermutated tumors would stand out, especially those harboring the DNA MMR deficiency [1]. This condition is known as Lynch syndrome and its ultra-antigenic activity is well documented ([1,2] and references therein). Such tumors stimulate the adaptive immune response by accruing 100–1000 times more mutations than typical tumors do, thus displaying higher antigenic activity. By releasing the brakes on T cells that become activated through recognition of the mutant proteins, Vogelstein *et al*. realized that Keytruda would make most tumors with MMR deficiency susceptible to immune attack, as it has been the case [1]. These crucial observations lead to the emergence of a novel therapeutic paradigm: The linking of drug-induced genomic instability with a checkpoint blocker. One example of this therapeutic mode is described in this forum. Another alternative also described in some detail requires combining Keytruda with an epigenetic drug modulating DNA methylation, a modality known to cause genomic instability. However, the latter approach is not tumor specific, as widespread demethylation opens up possibilities for catastrophic somatic consequences. By contrast, what is needed are tumor-specific methodologies, which will be proposed in this article.

In a recent clinical trial [2], researchers evaluated Keytruda in a cohort of 86 advanced cancer patients with 12 tumor types including colorectal, breast, prostate, and pancreatic cancer; all harboring the MMR deficiency. All cases were unyielding to standard tumor-specific treatment. After bimonthly dose of Keytruda, cancer remission was observed in the majority of the patients. In 18 patients, including two with advanced pancreatic cancer who had a dismal prognosis, complete responses were achieved, whereas objective radiographic responses were observed in 53% of patients. Responses were durable with median progression-free and overall survival limits not reached 3 years after the study was initiated. The data support the hypothesis that the significantly large antigenic activity in MMR-deficient cancers makes them hypersensitive to immune checkpoint blockade, regardless of the originating tumor.

The data in support of such striking conclusions were provided to the FDA months prior to publication and won Keytruda approval through priority review, enabling the monoclonal antibody to be used in any solid tumor on patients harboring the MMR deficiency[ii]. Spectacular as these findings are, the MMR deficiency phenotype is rare (although probably underdiagnosed) in cancer, with a 1–3% occurrence [1–3].

Towards a Drug-Induced MMR Deficiency Phenotype

The striking results described above and reported in [2] prompt the following question:

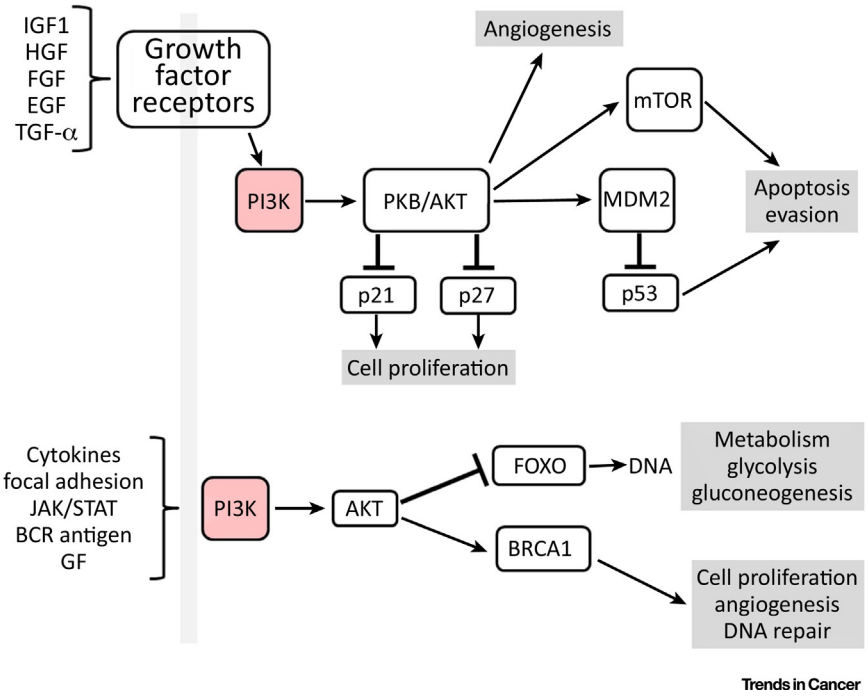

Figure 1. Simplified Scheme Highlighting the Role of PI3K As Signal Transducer for Cancer Pathways Recruited by Cancer Growth Factors (Upper Panel, Adapted from Pathway hsa05200, Kyoto Encyclopedia for Genes and Genomes, http://www.genome.jp/kegg-bin/show_pathway?hsa05200), and Metabolic Role in MMR-Associated Signaling Pathway (Lower Panel, Adapted from Pathway hsa04151, http://www.genome.jp/kegg-bin/show_pathway?hsa04151). EGF, epidermal growth factor; FGF, fibroblast growth factor; FOXO, forkhead box O; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; JAK, Janus kinase; MDM2, mouse double minute 2 homolog; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; STAT, signal transducer and activator of transcription; TGF, transforming growth factor.

Is it possible to induce the MMR deficiency in any solid tumor, for example through targeted drug-based therapy, and thereby enhance the cancer antigenic activity and diversity to turn the Keytruda-drug combination into a universal cure? In other words, is it possible to engineer drug-induced hypersusceptibility to Keytruda in cancers that are inherently impervious to checkpoint blockade?

The likely answer to this question is yes. The key to the problem resides in identifying a major signaling pathway recruited to promote MMR and finding a therapeutically relevant KI (kinase inhibitor) that would block that pathway. It turns out that the phosphoinositide 3-kinase (PI3K) is a key signal transducer for the MMR-associated signaling pathway [3], while acting as a major hub in cancer pathways recruited by tumor-secreted growth factors (Figure 1). Within MMR signaling, PI3K plays a metabolic role as it mediates carbohydrate uptake so that the antimetabolic role of PI3K inhibition is the depletion of nucleotides. This depletion is a consequence of reduced flux through glycolysis that results in a decrease in R5P required for base ribosylation [3] (Figure 1). Ultimately, the nucleotide depletion induces the DNA damage phenotype [3]. Thus, treatment with a PI3K inhibitor, specifically with a PI3Kα inhibitor [3], leads to nucleotide depletion in rapidly proliferating cancer cells that could result in DNA replication stress within hours. This metabolic effect is likely to be specific to tumor cells because they rely more heavily on *de novo* nucleotide biosynthesis than normal cells do. This tumor specificity as well as its central role in cancer-associated pathways (Figure 1) explains the therapeutic benefit of PI3K inhibitors.

The interference with DNA synthesis in tumor cells through nucleotide depletion induced by PI3K inhibitors results in replication stress, as tumor cells enter the S phase while DNA synthesis is compromised. Thus, nucleotide shortage leads to error-prone DNA replication, which in turn overloads the cell reliance on MMR mechanisms.

Concluding Remarks

As described above, treatment with PI3K inhibitors [3] is likely to realize the Vogelstein–Diaz scenario for enhanced antigenic activity and checkpoint hypersensitivity, mimicking the MMR deficiency condition and thereby making the tumor highly susceptible to an adaptive immune attack promoted by Keytruda. In light of this argument, the clinical evaluation of a combined Keytruda + PI3K inhibitor treatment constitutes an imperative in the quest for a definite universal cure for solid tumors.

Alternatively, desirable levels of susceptibility to checkpoint blockade may be obtained through combinations with epigenetic therapeutic agents. Inhibitors of DNA methyltransferase 1 (DNMT1) have been therapeutically exploited already since an array of tumor suppressor genes and DNA repair genes are frequently silenced in tumors through aberrant hypermethylation [4]. Most crucially, such DNMT1 inhibitors promote genomic instability through chromatin remodeling and compromise MMR and DNA damage repair (DDR) due to the DNMT1-associated recruitment of MMR and DDR pathways. As expected, a high mutational load is found to correlate well with DNMT1 suppression [4], promoting a phenotype of high immunogenicity akin to the Vogelstein–Diaz scenario. However, while combinations

of checkpoint immunotherapy with epigenetic therapeutic agents may also merit clinical evaluation, major caveats pertaining to the dangerous somatic impact of widespread DNA demethylation make epigenetic drugs less desirable promoters of the hypersusceptibility phenotype.

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Resources

[i]http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/125514s012lbl.pdf

[ii]https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm560167.htm

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