Table 1. Putative SZ Biomarkers Identified by the Chemical–Genetic Criterion

SZ Gene	Gene Name	Anti-SZ Drug	MoA
AKT[a]	V-akt murine thymoma viral oncogene homolog 1	Risperidone[d,e,f]	N/A
CACNA1C[a]	Calcium channel, voltage-dependent, L type, alpha 1C subunit	Celecoxib[e]	N/A
		Isradipine[e]	Inhibitor[e]
CCHCR1[a]	Coiled-coil alpha-helical rod protein 1	Allopurinol[e]	N/A
CHRM4[b]	Cholinergic receptor, muscarinic 4	Aripiprazole[e]; chlorprothixene[e]; clozapine[e]; olanzapine[e]; quetiapine[e]; ziprasidone[e]	Antagonist[e]
CHRNA7[c]	Neuronal acetylcholine receptor subunit alpha-7	TC-5280[d]	Inhibitor[d]
DPYD[a]	Dihydropyrimidine dehydrogenase	Methotrexate[e]	N/A
DTNBP1[a]	Dystrobrevin binding protein 1	Clozapine[d,e,f]; haloperidol[d,e,f]	N/A
HTR2A[c]	5-hydroxytryptamine receptor 2A	Mesoridazine[d]; olanzapine[d]; paliperidone[d]; quetiapine[d]; risperidone[a]; ziprasidone[a]	Antagonist[d]

[a]From [1].
[b]From [6].
[c]From [5].
[d]From the Therapeutic Target Database (http://database.idrb.cqu.edu.cn/TTD/).
[e]From ClinicalTrials (https://clinicaltrials.gov/).
[f]From DrugBank (http://www.drugbank.ca/).

link between genes, genetic diseases, and therapeutic drugs, we propose that the drug and its action might provide important information that could be used as a chemical–genetic criterion in facilitating the definition of disease biomarkers. Thus, the MoA of a drug might also yield useful insights into the pathogenic mechanisms of biomarker candidates.

Acknowledgements

This work was supported by the Natural Science Foundation of Hubei Province (grant 2013CFA016) and the Fundamental Research Funds for the Central Universities (grant 2662015PY004).

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http://dx.doi.org/10.1016/j.molmed.2016.04.001

References

1. Modai, S. and Shomron, N. (2016) Molecular risk factors for schizophrenia. *Trends Mol. Med.* 22, 242–253
2. Brinkman, R.R. *et al.* (2006) Human monogenic disorders - a source of novel drug targets. *Nat. Rev. Genet.* 7, 249–260
3. Wang, Z.Y. *et al.* (2012) Can medical genetics and evolutionary biology inspire drug target identification? *Trends. Mol. Med.* 18, 69–71
4. Plenge, R.M. *et al.* (2013) Validating therapeutic targets through human genetics. *Nat. Rev. Drug. Discov.* 12, 581–594
5. Wang, Z-Y. and Zhang, H-Y. (2013) Rational drug repositioning by medical genetics. *Nat. Biotechnol.* 31, 1080–1082
6. Nelson, M.R. *et al.* (2015) The support of human genetic evidence for approved drug indications. *Nat. Genet.* 47, 856–860
7. Griffith, M. *et al.* (2013) DGIdb: mining the druggable genome. *Nat. Methods* 10, 1209–1210
8. Sun, H-Y. *et al.* (2014) Finding chemical drugs for genetic diseases. *Drug Discov. Today* 19, 1836–1840
9. Law, V. *et al.* (2014) DrugBank 4.0: shedding new light on drug metabolism. *Nucleic Acids Res.* 42, D1091–D1097
10. Ripke, S. *et al.* (2011) Genome-wide association study identifies five new schizophrenia loci. *Nat. Genet.* 43, 969–976
11. He, K. *et al.* (2014) CACNA1C, schizophrenia and major depressive disorder in the Han Chinese population. *Br. J. Psychiatry.* 204, 36–39
12. Bigos, K.L. *et al.* (2010) Genetic variation in CACNA1C affects brain circuitries related to mental illness. *Arch. Gen. Psychiatry.* 67, 939–945

Spotlight

Learning from PD-1 Resistance: New Combination Strategies

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Only a minority of cancer patients respond to anti PD-1 immunotherapy. A recent study demonstrates that PD-1 therapy-resistant melanoma patients present distinct signatures of upregulated genes involved in immunosuppression, angiogenesis, monocyte and macrophage chemotaxis, extracellular matrix remodeling, and epithelial–mesenchymal transition (EMT). Combination targeting of these pathways with PD-1 may help overcome PD-1 resistance, thus producing effective antitumor immunity.

Blockade of either the PD-1 receptor or its ligand PD-L1 has improved overall survival in Phase III trials in patients with melanoma, non-small cell lung cancer, and kidney cancer. Early studies suggest that PD-1 pathway blockade may benefit a subset of patients in many other types of cancer. Nevertheless, the majority of patients fail to respond to PD-1 pathway blockade and insights into improving response rates are critically needed. In a recent report published in *Cell*, Hugo *et al*. analyzed the genomic and transcriptomic features of pretreatment tumor specimens to determine molecular signatures distinguishing response from resistance in

metastatic melanoma patients treated with PD-1 blockade immunotherapy [1].

Studies have supported the hypothesis that response to PD-1 blockade is associated with a smoldering, but ineffective, immune response, sometimes referred to as a 'hot' tumor, characterized by PD-L1 expression on tumor or infiltrating immune cells, $CD8^+$ T cell infiltration, and high neoantigen load. Somewhat surprisingly, Hugo *et al.* found no difference between primary responders and progressors in the expression of genes encoding PD-L1, PD-L2, or PD-1, or genes associated with activated T lymphocytes. This could be partly attributed to the fact that immune checkpoints, such as PD-L1, are expressed on infiltrating immune cells associated with both a good prognosis (dendritic cells) [2] and a poor prognosis (myeloid-derived suppressor cells). In addition, lack of differences in gene expression between groups could also be partly attributed to multiple strategies of immune evasion accumulated by tumor cells and infiltrating immune cells [3]. Using large-scale genomic analysis, several groups have found that the mutational burden or neoantigen load in tumors positively associates with a clinical benefit derived from therapeutic blockade using immune checkpoint inhibitors [4]. However, there has been a broad overlap in the number of mutations observed in responders and nonresponders. Here, the analysis provided by Roger Lo's group suggests that high neoantigen load does not predict a response to PD-1 immunotherapy, but rather, that it is associated with improved patient survival. Some genes were found to be more frequently mutated in the responder cohort. In particular, mutations in *BRCA2* were more frequent in tumors from the responding cohort. Loss of BRCA2-mediated DNA breakpoint repair function would not be expected to result in as large a number of neoantigens as tumors exhibiting mismatch repair deficiency. As such, BRCA2 is an example of a protein harboring mutations that can lead to potential neoantigens as well as loss of function, possibly driving tumorigenesis.

While the authors did not report a predictive upregulated gene signature associated with response to PD-1 immunotherapy, they did report a transcriptional signature associated with resistance to PD-1 immunotherapy, termed Innate PD-1 RESistance (IPRES). As illustrated in Figure 1, the IPRES signature includes genes involved in immunosuppression (*IL10*) and angiogenesis (*VEGFA*, *VEGFC*, *FLT1*, and *ANGPT2*), monocyte and macrophage chemotaxis (*CCL2*, *CCL7*, *CCL8*, and *CCL13*) and EMT (*AXL*, *ROR2*, *WNT5A*, *LOXL2*, *TWIST2*, *TAGLN*, and *FAP*). Consistent with this pattern, nonresponders presented lower expression of the *CDH1* (E-cadherin) gene, a marker of differentiated epithelia. Alternate analyses by gene ontology enrichment and gene set variant analysis (GSVA) scores also showed that nonresponding tumors were enriched for expressed genes associated with wound healing, angiogenesis, hypoxia, TGF-beta signaling, EMT, as well as cell adhesion and extracellular matrix organization. However, future studies are required to examine whether these pathways represent parallel patterns of response failure and/or whether they are interconnected in any way.

EMT is a normal process in embryonic development and wound healing. In wounds, the early acute inflammatory process produces a red, warm, inflamed injury, followed by scarring and repair of the tissue architecture, which are tightly regulated processes. These include the induction of angiogenesis, the suppression of potentially destructive inflammatory responses, and the resolution of tissue injury (wound closure). Tumors may utilize this process to increase tumor survival by increasing metastatic features and immunosuppression. Interestingly, Spranger *et al.* have shown that increased β-catenin signaling leads to poor infiltration of T cells into melanoma tumors [5]. Moreover, increased Wnt-GSK3β-β-catenin signaling can also promote EMT [5], perhaps connecting these mechanisms to IPRES. In a prior study, Lo's group compared the transcriptomic signatures of melanomas before treatment with MAPK inhibitors (BRAF monotherapy or dual BRAF and MEK inhibition) [6]. They found that, at the time of resistance to MAPK inhibition, the majority of tumors developed gain-of-function changes in NRAS and KRAS, as well as upregulated EMT-gene associated changes. Some of these tumors harbored high intratumoral T lymphocytes prior to treatment, but presented decreased infiltrating lymphocytes and increased M2 macrophage markers (CSF1R and CD163) after developing resistance to treatment. In the present study, Hugo *et al.* found that the signature of MAPK inhibition resistance bore many features in common with the IPRES signature. Furthermore, the IPRES signature could also be found in the TCGA database in a subset of tumors, including lung adenocarcinoma, renal clear cell carcinoma, pancreatic adenocarcinoma, and colon adenocarcinoma. Whether the IPRES signature is associated with resistance to PD-1 monotherapy in these cancers remains to be tested.

The link between activation in the RAS–RAF–MEK–ERK–MAPK pathway and reduced lymphocyte infiltration in tumors is not unique to MAPK inhibitor therapy. For instance, in breast cancer patients treated with standard chemotherapy, increased MAPK signaling has been associated with fewer tumor-infiltrating lymphocytes, while the presence of tumor-infiltrating lymphocytes has been associated with a better clinical outcome [7]. Since PD-1 blockade is an approved therapy for lung and kidney cancer, determining whether the IPRES signature is associated with resistance to PD-1 blockade in these patients is an important next step. The analysis of other tumor types is of particular importance since EMT status has been linked to an inflammatory tumor microenvironment in lung adenocarcinoma [8]. In addition, this microenvironment has been characterized by the expression of PD-L1/2, PD-1, TIM-3, LAG-3, B7-H3, BTLA, and CTLA-4, as