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Target mutation-driven drug discovery

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In the era of precision medicine, genetics is becoming a key factor for the choice of therapies. For decades, it has been intuitively thought that variability in drug response relies, at least partly, on gene variants that may affect the pharmacokinetics and pharmacodynamics of drugs. The most considerable evidences regard polymorphisms in genes encoding enzymes and carriers involved in the ADME processes. Nowadays, more attention should be paid to genetic variations in drug targets, especially in cancer and rare diseases.

Inherited or acquired gene mutations represent the basis of cancer development and resistance to treatment. For instance, the translocation between chromosomes 9 and 22, forming the so-called Philadelphia chromosome, produces the BCR-ABL fusion protein with constitutive tyrosine kinase activity, which is a major oncogenic event in chronic myelogenous leukemia (CML) [1]. Standard CML treatment includes the tyrosine kinase inhibitor (TKI), imatinib, but nonresponse or relapse is observed in a number of patients. Clinical resistance to imatinib is mainly due to ABL gene mutations or amplification [2]. Other TKIs may be used in such patients, including dasatinib, nilotinib, and bosutinib, which are active against many ABL mutants resistant to imatinib. However, patients carrying the p.T315I mutation are unlikely to respond to any of these drugs and should be switched to ponatinib, the only TKI with clinically relevant activity in ABL p.T315I mutants. Indeed, ponatinib was developed using computational and structure-based drug design to target both the native ABL kinase and the isoform carrying the p.T315I mutation [3]. A deep combined crystallographic and SAR analysis suggested that ponatinib builds a broad network of optimized molecular contacts with its target, rendering binding more resistant to any single point mutation [4].

About 80% of rare diseases are clearly related to gene mutations. Gene therapy is available for very few of these, and therapy mainly relies on the use of small molecular-weight molecules. Ideally, the drug should allow the mutated protein to recover normal function. Cystic fibrosis (CF) is one of the most common rare diseases, with a prevalence estimated at 7 per 100,000 individuals in Europe [5]. CF is caused by loss-of-function mutations in the CFTR (ABCC7) gene encoding a membrane carrier for anions in epithelial cells, which is critical for a correct excretion of chloride and/or

bicarbonate ions and hydration of epithelial luminal fluid. More than 2000 CFTR mutations are known and loss of CFTR function stems from various molecular mechanisms. Thus, mutations are classified in several categories, including mutations impairing CFTR synthesis, CFTR maturation and trafficking, CFTR channel opening, CFTR channel conductance, or excessive CFTR degradation [6-7]. Drugs have been developed to act specifically on mutant CFTR according to these mechanisms. Potentiators, including the first approved CF drug ivacaftor, are expected to stimulate the activity of CFTR mutants that are expressed at the cell surface. Thus, ivacaftor was first approved for carriers of the p.G551D mutant and then extended to other similar mutations; yet, as a monotherapy, it is suitable only for about 5-8 % of CF patients. Besides potentiators, correctors are drugs that are able to "correct" the misfolding of CFTR mutants allowing expression at the cell surface. The first approved corrector, lumacaftor, is effective in homozygous carriers of the p.F508del mutant, who represent about 45% of the CF patients. Because the p.F508del mutation impairs both expression and gating of the channel, lumacaftor is used only in association with ivacaftor (single-pill combination) to increase treatment efficacy, with however the drawback of reduced tolerability. The corrector tezacaftor is better tolerated and is suitable for p.508del in compound heterozygosity, still in association with ivacaftor. A triple therapy was then approved, comprising ivacaftor, tezacaftor, and the novel elexacaftor, a corrector that binds to a different site in the CFTR protein and provides additional cell surface expression of p.F508del. This allows boosting further the activity of this mutant and is particularly advantageous in heterozygous patients carrying pF580del with another mutation displaying minimal function. With such an arsenal, about 90 % of CF patients are eligible for a target treatment. Ongoing studies aim at increasing efficacy and tolerability as well as broadening the number of targeted CFTR mutants.

Another solution to improve the therapy of rare diseases may consist in the repurposing of approved drugs. Non-dystrophic myotonias are a group of rare diseases unified by the main clinical symptom, *i.e.*, muscle stiffness, but caused by various genetic defects [8]. Loss of function of the ClC-1 chloride channel encoded by the CLCN1 gene causes dominant or recessive myotonia congenita [9]. The two main molecular mechanisms consist in a reduced cell surface expression and an impaired gating of ClC-1 channels. Gains of function of the Nav1.4 sodium channel encoded by SCN4A are responsible for dominant paramyotonia congenita or sodium channel myotonia [10]. Impaired inactivation of mutants is the main cause of increased Nav1.4 channel activity. Reduced chloride currents or increased sodium currents both lead to increased sarcolemma excitability, that is the electrophysiological basis of clinical myotonia. Treatment of myotonic syndromes is symptomatic; sodium channel blockers approved for cardiac arrhythmias or epilepsy have been used off-label for decades because they reduce the abnormal firing of action potentials. After a successful randomized clinical trial, the antiarrhythmic mexiletine received orphan drug designation and further obtained full approval for myotonic syndrome. However, mexiletine does not fit to every patient due to suboptimal response or non-tolerability. We showed that Nav1.4 channel mutants with a positive shift in the voltage dependence of fast inactivation are less sensitive to mexiletine than wild-type channels in vitro [10]. In such a case, the antiarrhythmic flecainide may prove useful because the drug potency is less affected by the gating defect. These laboratory investigations were successfully translated to the clinic, since patients carrying p.G1306E or p.P1445L SCN4A mutations who were little satisfied with mexiletine obtained great benefits with flecainide [11-14]. As regards ClC-1 chloride channels, neither correctors nor potentiators are available [15]. Known ClC-1 blockers, such as the nonsteroidal anti-inflammatory drug niflumic acid, are being investigated for defining molecular binding sites with the aim of designing gating potentiators and as proof of concept for pharmacological chaperones able to promote cell surface expression [15]. Thus, similarly to CFTR in cystic fibrosis, ClC-1 mutation stratification in various

mechanistic classes will guide drug discovery for myotonic syndromes to obtain personalized therapies, with obvious advantages for treatment efficacy and safety.

Conclusion

We have reported a few examples of conditions in which the knowledge of target mutations has guided the development of personalized therapies. It is expected that the importance of target mutations in drug discovery will continue growing in the next future. This would not only apply to cancer and rare diseases but it would be relevant also for many other diseases, as target gene polymorphisms or mutations will be underscored. For instance, such a strategy would be likely crucial to success in the discovery of novel antivirals to tackle pandemics, such as that caused by COVID-19 variants [16].

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