Editorial

For reprint orders, please contact: reprints@futuremedicine.com

Pharmacogenomics

Prime time for doxorubicin-induced cardiotoxicity genetic testing

Tarek Magdy[1,2] & Paul W Burridge*[,1,2]

[1]Department of Pharmacology, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

[2]Center for Pharmacogenomics, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

*Author for correspondence: paul.burridge@northwestern.edu

> "Over the past 15 years, we have experienced significant advances in all aspects of the hiPSC field, including generation and characterization, expansion and scalability while maintaining pluripotency and genomic stability and differentiation into multiple lineages. This progress has allowed the wider use of hiPSCs in a variety of fields, including regenerative medicine, disease modeling, developmental biology and drug discovery."

First draft submitted: 14 March 2022; Accepted for publication: 14 March 2022; Published online: 5 April 2022

Keywords: cardiotoxicity • chemotherapy • clinical translation • human-induced pluripotent stem cells • pharmacogenomics

Toxicity to the heart is one the most serious potential side effects of anticancer therapies. Anthracyclines, exemplified by doxorubicin, are a family of cytotoxic agents that has been extensively used for more than 50 years and has proven highly effective in the treatment of a wide variety of cancers. Yet anthracyclines have a well-established side effect of causing dose-dependent cardiotoxicity. The most thorough data, using a well-established cutoff (reduction in left ventricular ejection fraction from >10% to less than 50%), shows that this anthracycline-induced cardiotoxicity (AIC) occurs in 9% of patients, on average after just 3.5 months, and nearly always (98%) within the first year after treatment [1].

AIC is a polygenic phenotype that involves several genes. So far, pharmacogenomic research has collectively identified more than 100 genome loci that are statistically associated with AIC [2]. The most statistically significant AIC-associated single nucleotide polymorphism (SNP) is rs2229774 in *RARG*. This SNP occurs in approximately 15% of the population and thus could contribute to the AIC predisposition in a substantial number of patients. The major limitation in applying the results of these many pharmacogenomic associations is that the mechanistic implication in AIC is typically ambiguous. To date, none of this genome-informed mechanistic insight has resulted in the development of anthracycline cardioprotective agents. There is one on-market, US FDA-approved cardioprotective drug, dexrazoxane, which, like anthracyclines, interacts with TOP2A, yet the exact mechanism of cardioprotection is still unknown.

Human induced pluripotent stem cells (hiPSCs) represent a uniquely powerful cellular model that can synergize pharmacogenomics research. In 2007, the first hiPSC lines were reported derived from fibroblasts cells by overexpression of four transcription factors: *POU5F1*, *SOX2*, *KLF4* and *MYC* (or *POU5F1*, *SOX2*, *NANOG* and LIN28) using integrating virus-based systems [3,4]. This work was followed by the generation of hiPSC without modification of the patient's genome [5,6] and from patients' peripheral blood [7].

Over the past 15 years, we have experienced significant advances in all aspects of the hiPSC field, including generation and characterization, expansion and scalability while maintaining pluripotency and genomic stability and differentiation into multiple lineages. This progress has allowed the wider use of hiPSCs in a variety of fields, including regenerative medicine, disease modeling, developmental biology and drug discovery.

The differentiation of hiPSCs is an area that has received substantial interest, especially cardiomyocytes. Highly advanced and robust methods for turning hiPSC into cardiomyocytes now exist, using chemically defined media and small molecules. These protocols work with cells in highly amiable monolayers or with cells in suspension generating ~100 million cardiomyocytes per batch [8,9]. Typical differentiation protocols produce 80–90% pure

Future Medicine

TNNT2^{+} cardiomyocytes [10] which can be enhanced up to 99% using cardiac lineage-restricted promoters such as *TNNT2*, *MYH7* or *NKX2–5* driving antibiotic resistance genes [8]. hiPSC-derived cardiomyocytes (hiPSC-CMs) have been used to study several cardiovascular diseases and phenotypes including long QT syndrome [11,12], LEOPARD syndrome [13], Timothy syndrome [14], arrhythmogenic right ventricular cardiomyopathy [15], dilated cardiomyopathy [16], Barth syndrome [17] and diabetic cardiomyopathy [18].

In 2016 we leveraged our advances in monolayer cardiac differentiation to demonstrate, for the first time, that patient-specific hiPSC-CMs recapitulate breast cancer patients' susceptibility to doxorubicin-induced cardiotoxicity (DIC) [19]. Here we showed that hiPSC-CMs are compatible with a wide array of high-throughput functional and biochemical assays that are suitable for *in vitro* DIC phenotypic characterization. This work proved that predisposition to DIC is genomic in nature.

This finding led us to wonder what is the role of the genome in DIC, and if the hiPSC-CM model could be used to discover novel cardioprotectants which resulted in our recent work demonstrating that hiPSC-CMs recapitulate specific SNP-dependent response to doxorubicin. In our recent work in *Cell Stem Cell* [20], we validated that "the GWAS-identified variant rs2229774 as directly causative in DIC, confirming that cells from patients harboring this variant are at higher risk of DIC. We discover that a small-molecule RARG agonist significantly attenuates cardiomyocyte doxorubicin sensitivity both in vitro and in vivo, reducing murine acute cardiotoxicity by almost 50%". To do this, six doxorubicin-treated cancer patients were recruited, of which three harbor the risk allele of SNP rs2229774 and suffered DIC (S427L), and three harbor the reference allele and did not experience DIC (control). First, we investigated the effect of doxorubicin on sarcomeric disarray, cell viability, caspase 3/7 activity as a marker for apoptosis, production of reactive oxygen species, phosphorylated H2A histone family member X (γH2AX) as a marker for DNA damage in our patient-derived hiPSC-CMs. These experiments demonstrated that cardiomyocytes derived from these patients recapitulate their risk to DIC and that S427L cardiomyocytes were significantly more sensitive to doxorubicin in comparison to control cardiomyocytes. Next, we moved on to confirm the role of *RARG* in DIC by generating isogenic hiPSC lines with *RARG* knockout and overexpression. Using our *in vitro* phenotypic characterization assays, we discovered that decreased RARG expression results in increased DIC.

We then investigated the effect of doxorubicin on the transcriptome profile of patient-derived cardiomyocytes. After treatment with doxorubicin, S427L hiPSC-CMs showed significantly more activation of doxorubicin-induced apoptosis, TP53 targets and oxidative phosphorylation than did control cells. In contrast, doxorubicin resistance and DNA repair were more significantly activated in control cells than in S427L cells. This finding was concordant with the *in vitro* DIC phenotypic characterization assays.

We next delved into the mechanism of DIC. DIC has been suggested to be mediated, at least in part, by *TOP2B* [21]. We demonstrated that RARG downregulates TOP2B and that this effect is reduced in S427L hiPSC-CMS, effectively allowing there to be more TOP2B and therefore more DIC. Interestingly, S427L, but not control cardiomyocytes, showed mitochondria membrane potential collapse that is mediated by lower expression of genes crucial for mitochondrial biogenesis downstream of *TOP2B*, including *PPARGC1A* and *PPARGC1B*. We observed significantly higher expression of cardioprotective phosphorylated ERK (pERK) in S427L hiPSC-CMs, potentially a response mechanism to the increased DIC. pERK regulates many genes involved in cardiomyocyte survival through the activation of the transcription factor, cyclic AMP (cAMP) response element binding (CREB) protein which controls cardiomyocytes survival signaling pathway.

To confirm the causality of the *RARG* genetic variant rs2229774 in DIC, we then used CRISPR-Cas9-mediated genome editing to introduce the CT rs2229774 variant allele into control patient-specific hiPSC lines that harbored the reference CC genotype (S427S) and did not experience cardiotoxicity after doxorubicin treatment. The introduction of the variant allele in control cells, increased their sensitivity to doxorubicin at all concentrations tested, as assessed by cell viability, mitochondrial membrane potential and DNA damage. These results confirm that the rs2229774 SNP alone is responsible for the increased risk of DIC.

Because SNP rs2229774 increases DIC via impairing RARG expression and function, we hypothesized that the activation of *RARG* signaling might rescue S427L cardiomyocytes from DIC. To test this hypothesis, we investigated the cardioprotective effect of three RARG-specific small-molecule agonists, BMS961, CD437 and CD1530, and the general retinoic acid receptor agonist all-trans retinoic acid (ATRA) against 10 increasing doses of doxorubicin. We showed that CD1530 was the strongest cardioprotective molecule among all screened molecules. Notably, CD1530 exerted its cardioprotective effect through the upregulation of RARG expression that in turn led to the repression

of TOP2B expression and the upregulation of mitochondrial biogenesis proteins. The CD1530 cardioprotective effect was then confirmed in murine in that mice treated with doxorubicin plus CD1530 showed significantly better cardiac function compared with mice treated only with doxorubicin. Importantly, we demonstrated that CD1530 did not attenuate the chemotherapeutic effect of CD1530 when tested in four different breast cancer cell lines.

This work demonstrates that patient-specific hiPSC-CMs recapitulate genetic variant-dependent DIC risk. Importantly, we showed that hiPSC-CMs are suitable for investigating DIC in a resolution that is high enough to detect the causality of SNP in relation to DIC. Nearly 48% of candidate drugs fail during phase I clinical trials because of the lack of efficacy. This percentage increases to 55% in phase II clinical trials in which more patients are normally recruited [22]. This indicates the necessity of a more human-relevant model that can complement the currently used animal, cellular, *ex vivo* models for the investigation of diseases molecular mechanisms and drug discovery. On the other hand, genomic association studies provide a wealth of genetic information that when combined with the preclinical studies represent a source of evidence that can increase the success rate of drug candidate during the drug discovery process [23,24].

Herein, using patient-derived heart cells, we demonstrated that SNP rs2229774 is a causal risk allele that increases the incidence of DIC. Moving from the mechanism to drug discovery, we showed that the *RARG* agonist CD1530 can protect against DIC. We believe that now is the prime time for the scientific community and for the authorized organizations to start considering the real translation of this discovery into the routine clinical practice. This work highly recommends that cancer patients treated with doxorubicin should be screened for SNP rs2229774 before starting the treatment so that we know the likelihood of a carrier patient to experience severe cardiotoxic adverse event beforehand. Similarly, the cardioprotective effect of CD1530 (or a derivative) should be tested clinically. In conclusion, patient-specific hiPSC-CMs represent a powerful platform that provides human-derived data ready for clinical application that we believe should be taken with a serious translational consideration.

Financial & competing interests disclosure

This work was supported by NIH NCI grant no. R01 CA220002 to PWB. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1. Cardinale D, Colombo A, Bacchiani G *et al.* Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation* 131(22), 1981–1988 (2015).
2. Magdy T, Burridge PW. Use of hiPSC to explicate genomic predisposition to anthracycline-induced cardiotoxicity. *Pharmacogenomics* 22(1), 41–54 (2021).
3. Yu J, Vodyanik MA, Smuga-Otto K *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318(5858), 1917–1920 (2007).
4. Takahashi K, Tanabe K, Ohnuki M *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5), 861–872 (2007).
5. Yu J, Hu K, Smuga-Otto K *et al.* Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 324(5928), 797–801 (2009).
6. Fusaki N, Ban H, Nishiyama A *et al.* Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.* 85(8), 348–362 (2009).
7. Chou BK, Mali P, Huang X *et al.* Efficient human iPS cell derivation by a non-integrating plasmid from blood cells with unique epigenetic and gene expression signatures. *Cell Res* 21(3), 518–529 (2011).
8. Burridge PW, Keller G, Gold JD, Wu JC. Production of de novo cardiomyocytes: human pluripotent stem cell differentiation and direct reprogramming. *Cell Stem Cell* 10(1), 16–28 (2012).
9. Kempf H, Zweigerdt R. Scalable cardiac differentiation of pluripotent stem cells using specific growth factors and small molecules. In: *Engineering and Application of Pluripotent Stem Cells.* Martin U, Zweigerdt R, Gruh I (Eds). Springer International Publishing, Cham, Switzerland, 39–69 (2018).
10. Peischard S, Piccini I, Strutz-Seebohm N *et al.* From iPSC towards cardiac tissue – a road under construction. *Pflugers Arch.* 469(10), 1233–1243 (2017).
11. Itzhaki I, Maizels L, Huber I *et al.* Modelling the long QT syndrome with induced pluripotent stem cells. *Nature* 471(7337), 225–229 (2011).

12. Malan D, Zhang M, Stallmeyer B *et al.* Human iPS cell model of type 3 long QT syndrome recapitulates drug-based phenotype correction. *Basic Res. Cardiol.* 111, (2016).
13. Carvajal-Vergara X, Sevilla A, D'Souza SL *et al.* Patient-specific induced pluripotent stem cell derived models of LEOPARD syndrome. *Nature* 465(7299), 808–812 (2010).
14. Yazawa M, Hsueh B, Jia X *et al.* Using iPS cells to investigate cardiac phenotypes in patients with Timothy syndrome. *Nature* 471(7337), 230–234 (2011).
15. Kim C, Wong J, Wen J *et al.* Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 494(7435), 105–110 (2013).
16. Sun N, Yazawa M, Liu J *et al.* Patient-specific induced pluripotent stem cells as a model for familial dilated cardiomyopathy. *Sci. Transl. Med.* 4(130), 130ra147 (2012).
17. Wang G, Mccain ML, Yang L *et al.* Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies. *Nat. Med.* 20(6), 616–623 (2014).
18. Drawnel FM, Boccardo S, Prummer M *et al.* Disease modeling and phenotypic drug screening for diabetic cardiomyopathy using human induced pluripotent stem cells. *Cell Rep* 9(3), 810–821 (2014).
19. Burridge PW, Li YF, Matsa E *et al.* Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat. Med.* doi:10.1038/nm.4087 (2016).
20. Magdy T, Jiang Z, Jouni M *et al.* RARG variant predictive of doxorubicin-induced cardiotoxicity identifies a cardioprotective therapy. *Cell Stem Cell* doi:10.1016/j.stem.2021.08.006 (2021).
21. Zhang S, Liu X, Bawa-Khalfe T *et al.* Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat. Med.* 18(11), 1639–1642 (2012).
22. Harrison RK. Phase II and phase III failures: 2013–2015. *Nat. Rev. Drug Discov.* 15(12), 817–818 (2016).
23. Hingorani AD, Kuan V, Finan C *et al.* Improving the odds of drug development success through human genomics: modelling study. *Sci. Rep.* 9(1), 18911 (2019).
24. Magdy T, Burridge PW. The future role of pharmacogenomics in anticancer agent-induced cardiovascular toxicity. *Pharmacogenomics* 19(2), 79–82 (2018).