A fishing trip to cure arrhythmogenic cardiomyopathy?

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Abstract: The paper entitled "Identification of a New Modulator of the Intercalated Disc in a Zebrafish Model of Arrhythmogenic Cardiomyopathy", as published in 2014 in *Science Translational Medicine*, examined the effects of the newly discovered drug SB216763 (SB21) on arrhythmogenic cardiomyopathy (ACM). In this paper, the authors focused on mechanisms underlying ACM and the accompanying molecular and cellular alterations. Most importantly they showed that SB21 was able to rescue and partly reverse the ACM phenotype in three different experimental models: (I) a zebrafish model of Naxos disease induced by the overexpression of the 2057del2 mutation in plakoglobin (PKG); (II) neonatal rat cardiomyocytes overexpressing the same mutation in PKG; (III) cardiomyocytes derived from induced pluripotent stem cells expressing two different forms of mutations in plakophilin-2. This editorial will focus on the potency and possible restrictions concerning SB21 treatment as a potential intervention for ACM and the usefulness of the applied zebrafish models in general.

Keywords: Arrhythmogenic cardiomyopathy (ACM); zebrafish; SB216763 (SB21); GSK-3β; therapy

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Arrhythmogenic cardiomyopathy (ACM) (also known as arrhythmogenic right ventricular cardiomyopathy/dysplasia, ARVC/D) is a progressive and primarily heritable heart disease that is caused by mutations in mainly desmosomal genes. Of those genes, mutations are most commonly found in the gene encoding plakophilin-2 (1). ACM is characterized by degenerative fibro-fatty replacement of cardiomyocytes, alterations of gap junctions and ion channels (especially Cx43 and $Na_v1.5$) (2,3), and redistribution of plakoglobin (PKG) from the junctions to the nucleus (4). In the last decade a vast amount of knowledge has been acquired about the etiology of this relatively rare disease and the difficult mode of diagnosis has been improved through a refined definition of the Task Force Criteria (5). Despite of that, the mechanisms that trigger the manifestation and progression of the disease with in particular the arrhythmogenic aspect are only at the onset of understanding. In the article of Asimaki and co-workers (6), the authors used three different experimental models that were able to recapitulate aspects of the disease as seen in patients, and by this, they accomplished to gain important insights into the underlying mechanisms.

The different models that were studied revealed several aspects of the disease as seen in patients. The zebrafish overexpressing the PKG 2057del2 mutation (causing Naxos disease) showed cardiomegaly, peripheral edema and a reduced cardiac output. This was accompanied with electrical remodeling (reduction of I_{Na}, I_{k1} and depolarization of the resting membrane potential) and 55% mortality when maturity was reached. Other molecular hallmarks of ACM, being a reduction of Cx43, $Na_v1.5$, and PKG at the membrane, were shown in the two *in vitro* models of cultured cardiomyocytes expressing clinically relevant mutated desmosomal proteins (PKG and plakophilin-2). Most notable in this study, through application of a library of 4,200 small molecules to the mutated zebrafish, the authors discovered a pharmacological tool that could prevent and even reverse the ACM phenotype with normalization of all the detrimental aspects listed above.

In the field of experimental cardiac research, more and more zebrafish models are used due to various advantages in comparison to other animal models. Mammalian and zebrafish hearts, for instance, share well-conserved cardiac

structures such as atria, ventricles, valves and even a cardiac conduction system (7). Although zebrafish hearts are very tiny, it is possible to record ECG's that show morphology, which is surprisingly similar to human ECG's. The first ECG's can already be taken at about 5 days post fertilization, where P and R waves can be seen that mirror atrial and ventricular depolarization respectively (8). Full ECG's are possible after 35 days post fertilization (9). Additionally, the Zebrafish's heart rate is much closer to the human heart rate than that of a mouse, thereby making it a suitable model to study, e.g., QT syndrome and arrhythmias (10,11). Furthermore, various action potential parameters are highly similar between humans and zebrafish. In both species, the upstroke during depolarization is dominated by sodium currents, the plateau phase by L-type calcium channels, and the repolarization by the rapidly activating delayed rectifier potassium currents (I_{Kr}). The latter plays a prominent role in drug induced arrhythmias and is, for example, not present in mice (12).

Besides these benefits, zebrafish models are popular to perform large library screenings, as also applied in the currently discussed study, and proof of principle experiments. The fish are inexpensive, have a high fertility (female fish can produce approximately 200 eggs a week) and show many advantages like small size, short generation time (most organs including a contracting heart tube are developed within 24 hours post fertilization), transparency during development, genetic tractability and the ability to survive without a functional cardiovascular system during development (7,13,14). Of course there are still some disadvantages compared to mammalian models like the mouse, one of them being the poor availability of antibodies that specifically recognize the proteins in fish (13).

The drug that was selected through the screen in zebrafish, SB216763 (SB21), is an inhibitor of the glycogen synthesis kinase GSK-3β and therefore an activator of the canonical Wnt pathway. Canonical Wnt pathways play an important role in various cellular processes, such as axis duplication, cell transformation, cardiac development and differentiation, cell-cell adhesion and hypertrophy (15). All canonical Wnt pathways include the β-catenin as a downstream substrate. Beyond the central role of GSK-3β, also the involvement of SAP97 is an intriguing finding since localization of this protein was not only found to be disturbed in ACM patients, but it is also associated with anchoring of $Na_v1.5$ and I_{k1} channels in the intercalated disk. The data in this study suggest that during development of the disease mislocalization (maybe due to trafficking defects) of these proteins is downstream of GSK-3β and that, similar as in patients, increased stretch of the cardiomyocytes/myocardium triggers a change in molecular signaling. Prevention of mislocalization or restoration of these proteins in the intercalated disk as mediated by SB21 therapy might have serious anti-arrhythmic consequences.

SB21 administration holds significant promise as therapy for ACM, a disease without an appropriate treatment yet. However, care should be taken when for instance children at risk to develop the disease (mutation carriers) are treated, because of the important role of canonical Wnt pathways in development and its various targets in the heart and other organs. Other aspects that demand attention besides the off target effects, include of course the systemic consequences of chronic treatment (given the ubiquitous role of GSK-3β) and to answer the question whether reversibility of the diseased myocardium still holds promise if the myocardium already shows aspects of fibro-fatty replacement, a common observation during progression of the disease in ACM patients.

Finally, although the zebrafish model will certainly not completely replace experimental mammalian models like the mouse in the future, the study by Asimaki *et al.* (6) shows that the model already offers considerable advantages, leading in combination with appropriately chosen additional model systems, to the discovery of a potential therapy for ACM.

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