Mouse models in arrhythmogenic right ventricular cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an important cause of ventricular arrhythmias and sudden cardiac death, especially in the young and in athletes (Marcus et al., 1982; Thiene et al., 1988; Basso et al., 2009). Mutations in one or more genes encoding desmosomal proteins are found in ≈50% of patients (McKoy et al., 2000; Rampazzo et al., 2002; Gerull et al., 2004; Pilichou et al., 2006; Syrris et al., 2006). Desmosomes are highly conserved structures that, together with adherens junctions, gap junctions, and desmosomes, end at the level of the intercalated disks and thereby play a crucial role in maintaining proper myocardial function (Delmar and McKenna, 2010). Recent findings indicating the presence of mixed type junctions (the area composite; Franke et al., 2006) and crosstalk between protein complexes pertaining to the different types of junctions (Delmar, 2004; Meadows and Isom, 2005; Safitz, 2005; Tavora et al., 2009; Li and Radice, 2010; Sato et al., 2011) have markedly changed the perception of the intercalated disk. Altered junctional organization, as the result of mutations, is thought to lead to myocardial damage and replacement fibrosis, the classical histopathologic pattern of ARVC (Basso et al., 2009). In advanced stages of the disease, focal scars cause electrical isolation of cardiomyocytes within non-conducting fibrous tissue, resulting in slow conduction and delayed activation, thus forming the substrate for re-entrant circuits and ventricular electrical instability. Over the years several mouse models have been developed to investigate the mechanisms of disease development in ARVC (Pilichou et al., 2011), focusing on four of the desmosomal proteins: Plakophilin-2, Plakoglobin (γ-catenin), Desmoplakin, and Desmoglein 2. All four will be discussed in detail below; an overview of all the models is given in Table 1.

PLAKOPHILIN-2 TARGETED DELETION

The first mouse model described that addresses disruption of one of the desmosomal proteins is the study by Grossmann et al. (2004) that describes the targeted deletion of plakophilin-2. The heterozygous mice carrying one wild type copy of plakophilin-2 were completely viable without any cardiac phenotype. Mouse homozygous for the deletion (Pkp2−/−) died during embryonic development around day 11 post fertilization. Embryos at day 10.5 appeared pale with blood aggregates in the interperitoneal cavity indicating the presence of small holes in the endothelial layers lining the heart and vessels. It would be interesting to see if the Pkp2−/− mice develop an ARVC-like phenotype upon exercise.

PLAKOLOBIN TARGETED DELETION AND OVEREXPRESSION MODELS

The first papers on mouse models involving a desmosomal protein described the targeted deletion of Plakoglobin (Pg) by two independent groups in 1996 (models Pg1 and Pg2; Bierkamp et al., 1996; Ruiz et al., 1996). Homozygous targeted deletion of Plakoglobin leads to embryonic lethality between embryonic day 9.5 and 16 due to cardiac malformations: thin cardiac walls and less trabeculation and frequent burst of the epicardial wall; in addition mice showed a blistering skin phenotype. Heterozygous animals appeared healthy and fertile. However, closer inspection of these mice (model Pg2) after the link between ARVC and desmosomal proteins had become clear showed that Pg2−/+ mice at 10 months after birth had enlarged right ventricles, increased spontaneous ventricular arrhythmias and right ventricular conduction slowing. No replacement fibrosis and remodeling of the junctions was observed, Cx43 localization and distribution were normal on immunofluorescence microscopy. All observed changes were exacerbated and expedited when mice were subjected to exercise.
Table 1 | Arrhythmogenic right ventricular cardiomyopathy mouse models.

| Nr | Protein      | Gene     | Mutation/targeted exons | Model type                      | Reference                                      |
|----|--------------|----------|--------------------------|---------------------------------|-----------------------------------------------|
| Pk1 | Plakophilin-2 | Pkp2     | Exon 1–intron 1          | Targeted deletion               | Grossmann et al. (2004)                       |
| Pk2 | Plakophilin-2 | Pkp2     | Exon 1–intron 1          | Targeted deletion               | Grossmann et al. (2004)                       |
| Pg1 | Plakoglobin  | Jup      | Exon 2–5                 | Targeted deletion               | Bierkamp et al. (1996)                        |
| Pg2–Pg4 | Plakoglobin | Jup      | Exon 34                  | Targeted deletion               | Ruiz et al. (1996), Kirchhof et al. (2006), Fabritz et al. (2011) |
| Pg5 | Plakoglobin  | Jup      | Exon 2–3                 | Inducible deletion; αMHCcre induced; αMHCMerCreMer tamoxifen induced double deletion | Li et al. (2011)                             |
| Pg6 | Plakoglobin  | Jup      | Exon 2–3                 | Flag tagged transgene           | Lombardi et al. (2011)                        |
| Pg7 | Plakoglobin  | Jup      | Wild type                | Truncated                        | Lombardi et al. (2011)                        |
| Pg8 | Plakoglobin  | Jup      | 2365Δ2                   | Transgene                       | Lombardi et al. (2011)                        |
| Dp1 | Desmoplakin  | Dsp      | Δ281–473                 | Targeted deletion with extra-embryonic rescue | Gallicano et al. (2001)                       |
| Dp2 | Desmoplakin  | Dsp      | Exon 2                   | Inducible deletion; αMHCcre induced | Garcia-Gras et al. (2006), Gomes et al. (2012) |
| Dp3 | Desmoplakin  | Dsp      | Wild type                | Flag tagged transgene, αMHC promoter | Yang et al. (2006)                           |
| Dp4 | Desmoplakin  | Dsp      | R2834H                   | Flag tagged transgene, αMHC promoter | Yang et al. (2006)                           |
| Dp5 | Desmoplakin  | Dsp      | Q90R                     | Flag tagged transgene, αMHC promoter | Yang et al. (2006)                           |
| Dp6 | Desmoplakin  | Dsp      | V30M                     | Flag tagged transgene, αMHC promoter | Yang et al. (2006)                           |
| Dg1 | Desmoglein 2 | Dsg2     | Wild type                | Flag tagged transgene, αMHC promoter | Pilichou et al. (2009)                       |
| Dg2 | Desmoglein 2 | Dsg2     | N271S                    | Flag tagged transgene, αMHC promoter | Pilichou et al. (2009)                       |
| Dg3 | Desmoglein 3 | Dsg2     | Exon 4–6                 | Targeted deletion               | Krusche et al. (2011), Kant et al. (2012)     |

Training (model Pg3; Kirchhof et al., 2006). Load reducing therapy is able to prevent these symptoms of ARVC in Pg± mice (model Pg4; Fabritz et al., 2011).

To circumvent the problem of neonatal lethality, a cardiac specific targeted deletion of Pg was developed under the control of αMHCCre. PgΔf f MHCcre (model Pg5) mice have ~30% of the WT protein as measured by Western blot, no Pg was detectable by immunofluorescence on cardiac sections. Phenotypically these mice largely recapitulate the human ARVC phenotype: cardiac sudden death, progressive dilation, and fibrosis in the cardiac walls (both in the left and the right ventricle) from 2 month onward. No cardiac fat deposition was observed. With transmission electron microscopy the structure of the desmosomes seemed to be disrupted: other desmosomal proteins (e.g., Dsg2) appeared to be absent from the intercalated disk (Li et al., 2011). Cell death in the PgΔf f MHCcre mice was at least partially through myocyte apoptosis in addition to myocyte necrosis in contrast to mice overexpressing N271S-Dsg2, which mainly showed myocyte necrosis (see below; Pilichou et al., 2006; Li et al., 2011). Interestingly, increased β-catenin staining was observed at the intercalated disk suggesting partial rescue by this close relative of Pg (γ-catenin). To test whether the lack of fast spontaneous death in these mice (model Pg5) was due to a partial rescue by β-catenin, double-targeted mice were created, carrying both a floxed Pg gene and a floxed β-catenin locus (PgΔf f; β-cateninΔf f). Crossing with αMHC/MerCreMer mice and subsequent tamoxifen injections effected specific targeted deletion. Double-targeted mice (model Pg6) showed a strong arrhythmogenic phenotype, with 100% of the double-targeted animals dying of sudden cardiac death between 3 and 5 months after tamoxifen injections. In contrast to either single targeted deletion and wild type littermates of which 4–9% died within 6 months of tamoxifen injection (Swope et al., 2012).

Two lines overexpressing wild type (model Pg7) and mutant (model Pg8) Pg were generated (Lombardi et al., 2011); both showed similar levels of increased incidence of sudden cardiac death, an indication that even moderate levels of overexpression of Pg disturb the balance of the mechanical interaction and signaling functions of Pg independent of the introduced truncating mutation.

**DESMOPLAKIN TARGETED DELETION AND OVEREXPRESSION MODELS**

Desmoplakin (Dsp) targeted deletion mice DspΔf f–/– die at embryonic day 6.5 of malformations in the extra-embryonic tissue before assessment of a cardiac phenotype is possible (Gallicano et al., 1998). To overcome this problem the extra-embryonic phenotype was rescued by tetraploid aggregation (model Dp1). The resulting embryos die around embryonic day E11. At E10 they show severe cardiac malformation although desmosomal-like structures appear to be present by transmission electron microscopy. As these mice die well before birth they are obviously unsuitable as a proper model for ARVC (Gallicano et al., 2001). The embryonic lethality of the DspΔΔ/ΔΔ mice is partially circumvented in the cardiac specific, αMHCCre induced, targeted deletion of Dsp (model Dp2; Garcia-Gras et al., 2006). The homozygous cardiac specific deletion of Dsp leads to embryonic growth arrest at day E10–E12 with embryos that appeared very pale, with no circulating red blood cells in organs. The heart was poorly formed with no chamber specification, 80% of DspΔ/Δ mice did not survive until delivery. Mice that did survive the embryonic period died within 6 weeks...
after birth. Heterozygous Dsp^+/− mice developed normally, however adult Dsp^+/− mice had thin ventricular walls, increased left ventricular diameters and reduced left ventricular ejection fraction. Spontaneous arrhythmias were observed on surface ECG and 4/5 mice developed ventricular arrhythmias after a single ventricular extra stimulus.

A more detailed study of these Dsp^+/− mice at earlier stages of disease development, when no abnormalities at the surface ECGs could be detected and no evidence of replacement fibrosis could yet be found, showed a significant increase in the activation time and inducible arrhythmias in Langendorff perfused hearts. These results indicate the presence of electrophysiological abnormalities before the onset of overt structural changes (Gomes et al., 2012). However, no electron microscopy was done to exclude the presence of disrupted desmosomes at the ultrastructural level.

Transgenic mice with cardiac overexpression of flag tagged human Dsp cDNA both wild type (WT-Tg, model Dp3) and with a C-terminal mutation (R2834H-Tg, model Dp4) were generated; the murine N271S mutation is available from their wild type littermates and Wt-Tg mice. R2834H-Tg hearts showed increased apoptosis and fibrosis along with reduced ventricular function and dilatation of both right and left ventricles. Co-immunoprecipitation indicated the disruption of the interaction between Dsp and Pg. at the ultrastructural level widening of the intercalated disk was observed in Dp4 mice.

**DESMOogleIN OVEREXPRESSION MODELS**

Transgenic mice with cardiac overexpression of flag tagged Dsg2 both wild type (Tg-WT, model Dg1) and with N271S-Dsg2 mutant (Tg-NS, model Dg2) were generated; the murine N271S mutation is the mouse homolog of the human ARVC mutation DSG2–N266S. Overexpression of Dsp with N-terminal mutations, e.g., V30M (model Dp5) and Q90R (model Dp6) led to embryonic lethality after embryonic day 13.5 due to reduced ventricular wall thickness and ventricular dilatation. R2834H-Tg mice had an increased heart weight/body weight ratio compared to both wild type littermates and Wt-Tg mice. R2834H-Tg hearts showed increased apoptosis and fibrosis along with reduced ventricular function and dilatation of both right and left ventricles. In conclusion, since the discovery a little over a decade ago that the cause of ARVC lies within the cardiac desmosomal complex and its associated proteins, a wealth of knowledge has been built up on the etiology of the disease. The use of the murine transgenic and targeted models has played a pivotal role in this process. The quest to answer the many remaining questions on the first stages of the disease and the search for a good treatment will continue during the next decade, the developed mouse models will undoubtedly play an important role in this process.
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