Editorial

Stem Cell INVESTIGATION

Nicotinamide, iRPE-in-a dish, and age-related macular degeneration therapy development

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Age related macular degeneration (AMD) is a complex retinal disorder, which progressively affects central vision in 4% of the population over 60 (1,2). AMD is caused by multiple environmental and genetic risk factors. Most importantly, smoking as well as genetic variants in the *CFH* gene (chrom. 1) and the ARMS2/HTRA1 locus (chrom. 10) contribute to the disease pathogenesis. Molecular modeling and functional annotation implicated oxidative stress, the innate immune response, lipid metabolism and neovascularisation in the pathobiology of AMD (1,3).

A common, dry form of AMD exist, and a, more rare, wet form (affecting 10% of patients). The dry form is characterized by the appearance of drusen underneath the retinal pigment epithelium (RPE), pigment atrophy and slow progressive loss of vision. There is no cure for this type of AMD, although dietary antioxidant, zinc or fish-oil supplements may postpone or even slow down the progression of the disease. The wet form of AMD usually appears even later in the life and consist of new fragile and leaky choroidal blood vessels growing through the blood-retina barrier, into the retina, which causes local fibrosis, scar tissue and vision loss. There is no effective cure for this type of AMD either, although bimonthly intra-ocular injections with anti-VEGF drugs (Lucentis, Avastin) may prevent further neo-vascularization (1,3).

Finding a cure and therapeutic research into AMD has been hampered so far by the lack of suitable experimental models: Only a few immortalised RPE cell lines, such as "ARPE-19 (ATCC)", have been available for *in vitro* studies, while there is no single animal model yet that captures all the complex AMD features at once. However, this is about to change: The recent introduction of stem cell and Crispr-cas9 technology will provide a wealth of new opportunities to model and study genetic disorders, including AMD.

A sneak preview of these new developments was published recently. In the May issue of *Cell Stem Cell*, Saini and coworkers published pioneering work and showed the extra-ordinary potential of human IPSCs and RPE modeling for therapeutic investigations in AMD (4). In summary, the authors selected patients, diagnosed with AMD and controls on the basis of their phenotype and ARMS2/HTRA1 risk genotypes (mostly homozygote risk *vs.* homozygote protective alleles). From these selected patients and controls, they constructed iPSCs and subsequently induced RPE (iRPE) cell lines. Next, they treated these *in vitro* models with Nicotinamide (NAM), shown to be effective against cognitive loss in Alzheimer's disease. Using RT-PCR, immunohistochemistry, RNA-seq and ELISA, the authors measured an increased expression of complement and inflammatory factors in the high-risk allele AMD samples compared to low-risk controls. Moreover, the authors observe that NAM treatment inhibits drusen-, complement factor and inflammatory protein formation. The authors conclude: "*NAM ameliorates the disease phenotype and may be an effective agent in developing therapies for AMD*" (4).

The latter statement may be a little optimistic, however, and multiple improvements for AMD modeling must still be implemented. The most fundamental question is, of course: does the iRPE-in-a-dish, developed from an aged fibroblast from a patient, reflect the AMD phenotype? Or do we look actually only at physiological age-related changes? [which, in mice, are also reduced by NAM (5)]. In the classical view, the RPE, including the Bruch's membrane (BM), was the only pathological site to be considered in AMD. However, more recent data strongly suggest a much more active role of the choroid, photoreceptors, and the blood system in the development of AMD than previously thought (6-8). A main stream AMD hypothesis is that nonpathalogical age-related retinal changes insidiously turn in into pathological (dry) AMD changes, while a more sudden twist on top of the ongoing pathology causes (wet) AMD (9,10). Thus, culturing RPE by itself may be a too simple model to use in AMD: more advanced *in vitro* models with appropriate controls taking a number of these extra-RPE aspects into account are under development (11), and could preferably be used in combination with the IPSCs-RPE approach.

Furthermore, Saini and coworkers [2017] (4), if they'd started their studies now, preferably would have used only male patients' fibroblasts. X-linked inactivation in females appears to be one source of variability in IPSCs development (12,13). Also, at least one sex-specific susceptibility locus for AMD has been identified (14). Although Blenkinsop and coworkers (15) found that stem cell derived RPE essentially reflects the key features of native RPE, we recently also found that there's still considerable non-sex influenced whole genome variation between stem cell derived RPE and native RPE (16). These more or less contrasting data, may results from the fact that there are now multiple, slightly different, protocols to derive RPE from stem cells (17-20). Indeed, this calls for more international standardization of stem cell RPE differentiation protocols.

On the other hand: do these potential molecular and cellular differences between (i)RPE also reflect relevant functional differences? We think not necessarily: as a pilot, we recently injected functionally RPE-deficient RPE65$^{-/-}$ mice subretinally with dissociated cells from the ARPE19 cell line, a frequently used RPE cell line resembling native RPE in some functionalities. In contrast to our expectations, we observed, after 6 months OCT and ERG follow-up studies, some functional ERG recovery in a few mice (21).

Saini and coworkers [2017] (4) further used, in their study, a relatively low amount of samples and patients per group. Apart from the defined alleles at the ARMS/HTRA1 locus, one can assume considerable (epi-) genetic variability at other AMD susceptibility loci and elsewhere in the genome between the samples (partly described). The combination of low number of samples and genetic variability made it subsequently necessary to choose very low stringency statistical tests and parameters (one-sided Fisher, FDR >0.1 and FC >2.0) for their comparative patho-biological read-outs. Nonetheless, the authors did find some interesting leads, confirming the existing hypothesis that the oxidative stress, complement and immune response pathways are intrinsically linked in the pathobiology of AMD (22). However, whether their specific results are relevant for AMD therapy, needs to be further investigated.

In their concluding statement, the authors propose that "NAM may provide an effective therapeutic opportunity for AMD" (4). This statement is probably not only based on their own results, but also on the overlap between the molecular pathology of AMD, atherosclerosis and Alzheimer's disease (10,23), where NAM is successfully under clinical investigation. While their (4) initial molecular and cellular results are indeed encouraging, the future medical applicability of NAM depends on many other factors yet to be investigated, including NAM (*in vivo*) working spectrum: aging versus AMD, efficacy in improved preclinical designed ocular studies, safety, ocular delivery methods, competition with other compounds/drugs, applicability for other diseases, intellectual property opportunities, regulatory requirements, cost, profits, medical specialists acceptance, available distribution networks, etc. As is normal in drug development land, only a very small amount of "promising candidate compounds" entering the medical development pipe-line, will actually turn out be the "wonder drug".

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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