Preclinical models in ophthalmic research

Purposeful perception of the local environment through solar light transmission has developed in many metazoans, starting from starfishes, clams and jellyfishes (1,2). Starting from very simple structures, including only light-sensing pigments, more complex eyes have been generated, endowed with different components to allow a fine perception of the surroundings, necessary for a better survival of the organism in its own specific environment (1). However, light is a strong energetic source, and the process of vision consumes a high amount of metabolic resources, finally generating catabolites and free radicals. With time, and in the absence of an equally strong scavenging protection, damage may accumulate at the level of the ocular structures and generate different types of pathologies. Moreover, certain conditions—such as premature birth or diabetes—may aggravate the speed at which tissue damage is produced, thus resulting in a faster development of certain pathologies. Retinopathy of prematurity (ROP) is a potentially blinding ocular disorder characterized by abnormal retinal vessel growth in premature infants in which the still incomplete retinal vasculature at birth represents the prerequisite for ROP development (3). Diabetic retinopathy (DR) is a diabetes complication characterized by retinal vessel proliferation that eventually leads to blindness if left undiagnosed and untreated (4). Age-related macular degeneration (ARMD) is a retinopathy involving the retinal pigmented epithelium (RPE) and the Bruch's membrane on which it rests, and that makes a permeable barrier with the choroid. When too many catabolites accumulates at this level, an inappropriate activation of the complement system may occur, which produces inflammation and damage, finally resulting in macular edemas, rupture of the Bruch's membrane and capillary invasion of the retina (5). Primary open angle glaucoma (POAG) is another type of retinopathy, belonging to the slow-progressing neurodegenerative diseases, due to the apoptotic death of retinal ganglion cells [forming the optic nerve (ON)] (6). The elevated intraocular pressure (IOP) is recognized as one of the major risk factors, together with vascular dysregulation, but by themselves they are not a necessary cause of glaucoma, which may insurge and progress despite specific therapeutic treatments (7). Therefore, neuroprotection is more and more emerging as a necessary complement to integrate the hypotonising therapy (8).

According to the 2019 WHO report (9), globally, at least 2.2 billion people have a vision impairment, among which at least 1 billion have a disease that could have been prevented or has yet to be addressed. In the same report, the prevalence of the major eye pathologies (excluding cataract and refractive conditions) are estimated worldwide at 196 million for ARMD, 146 million for DR and 76 million for POAG. In most developed countries, these three pathologies represent roughly the 85% of all ocular affections. To date, no cure exists for the different types of retinopathies, and the best that can be done is to slow down their progression with different therapeutic strategies. Ocular tumors, and more specific uveal melanoma, are eye pathologies that, beside the impairment of vision and the risk of enucleation, also pose a serious risk on patient's survival, due to their high metastatic propensity (10).

Therefore, in order to study the molecular mechanisms of these eye pathologies and to experiment new treatments, the availability of specific model systems—*in vitro* and even better *in vivo*—is a necessity for the improvement of our understanding of this matter, and the finding of new and more effective therapeutic approaches. Along this line, the goal of this special series has been to collect a series of practical interventions from scientists expert of such model systems, in order to allow those interested to set up their model of interest and run useful experiments.

The model presented by Lucchesi and Marracci (11) deals with the use of Muller cells cultures in vitro. Muller glial cells envelop all retinal neurons and extend through all retinal tissue layers. They strongly contribute towards the maintenance of retinal homeostasis, including the control of angiogenesis, and retinal blood flow regulation. Muller cells support cell protection and survival, through the secretion of neurotrophic factors and antioxidants, and the blunting of glutamate excitotoxicity. However, sustained Muller cells gliosis is more than often associated to almost all retinal diseases, triggering neuronal degeneration and retinal cell death by apoptosis (12). The paper by Lucchesi and Marracci describes how to use the Muller cell line MIO-M1 to study their response to stress conditions such as oxidative stress (OS) (cultured with hydrogen peroxide), pathological neovascularization (cultured with VEGF), hypoxic or hyperoxic conditions (cultured in low or high oxygen chamber). Moreover, they tell us how to derive primary Muller cell cultures from explanted retinas of normal or pathologic mice.

New vessels growth in the retina is the serious hallmark of blinding retinal pathologies, such as proliferative DR,

neovascular macular degeneration and retinopathy of the premature. The paper by Rossino (13) tells us how to culture retinal explants to be used as *ex vivo* experimental models to investigate the molecular mechanisms involved in neurovascular diseases, or to test in a short time the neuroprotective effects of molecules that could be used to prevent the toxicity of metabolic stressors. Along the same line, but shifting to an *in vivo* situation, the contribution of Canovai (14) presents different animal models of oxygen-induced retinopathy (OIR), which allow the quantification of abnormal neovessels and are susceptible to electrophysiologic, histological and molecular analyses. Finally, two different animal models of ARMD are described by Kim and Qian (15). Interestingly, the subretinal administration of linoleic acid peroxide (HpODE) may induce acute local degeneration of RPE and photoreceptors around and at the injection site, so that such degeneration expands peripherally through all retinal layers, finally inducing choroid neovascularization (CNV) as it happens in the neovascular form of ARMD. I want to cite here another model, however of atrophic ARMD, obtained through the subretinal injection of polyethylene glycol (PEG) (16), which could not find space in this series. Also, no animal models of DR are here described, although several exist. I think useful to mention here the most classical model, obtained after injection of streptozotocin in the rat (17), and also a spontaneous genetic model of DR in a special strain of rats named Torii (18), which allowed to address the therapeutic effects of anti-inflammatory and anti-angiogenic treatments.

Glaucoma is the next big issue among ophthalmic diseases. Two articles in this series address two different models of glaucoma, both based on the damage inflicted to the ON by the elevation of IOP. In the paper contributed by Maurizio Cammalleri (19) the compression of the ON made with the aid of forceps mimics the compression exerted on the ON at the level of its exit from the eye globe through the lamina cribrosa, in presence of severely elevated IOP (20), such as it may happen in acute closed angle glaucoma. Two more different models, somewhat closer to the human situation, are described in the paper by Rosario Amato (21). The first model is based on the intracameral injection of methylcellulose (MCE), which partially clogs the trabecular meshwork, thus decreasing aqueous humor (AH) outflow and inducing a moderate, long standing increase of the IOP, which in turn triggers all the kind of damages to retinal cells as observed in glaucoma. The model is well reproducible, and allows in a short time (a couple of weeks) to study the effects of treatments aimed at the neuroprotection (either direct or indirect) of RGC. The second model exploits a genetic variant in the mouse strain DBA/2J which, together with several different physiologic alterations, also shows a propensity over time to develop elevated IOP and glaucoma. However, only a fraction of the animals develop overt glaucoma, which takes many weeks to produce its effects on the ON.

The importance of light-induced phototoxicity has gained attention in the last years considering that light exposure induces photoreceptor degeneration and retinal dysfunction, a feature that is mimicked in the model of light-induced retinal damage. The work of Canovai (22) presents an easy and reproducible model to study the damage which can be inflicted to the retina by continuous exposure to bright light, and how photo-oxidative insults to retinal cells could be blunted by the use of certain natural antioxidant molecules.

Among the hereditary eye diseases, one missing issue concerns the mouse models of retinitis pigmentosa, a progressively blinding disease of the young age, with no treatment so far. Some animal model systems have been described that mimic this pathology, and I think useful to give some reference in order to fill this gap (23,24). Other gaps to be filled are those regarding dry eye and cataract, two main and widely diffused ocular pathologies, which however do not pose too much serious health problems. Several models of dry eye have been described over time, and recent collections of papers are here indicated (25-27). The genetic predisposition to cataract is a relatively recent issue, dealt with in this comprehensive review by Wada *et al.* (28).

Finally, the issue of ophthalmic tumors is dealt with in the article by Lehrmann and collaborators (29). In their review, they give a comprehensive description and summary of currently available preclinical models of the three main tumors in ophthalmic oncology: conjunctival melanoma (CM), uveal melanoma (UM), and retinoblastoma. A wide variety of *in vivo* model systems focusing on uveal melanoma are illustrated in the paper by Uner *et al.* (30). Mouse, rat, rabbit and hamster models are described, either spontaneous or induced by grafting of exogenous tumor cells. The issue of metastatic spreading of such tumors is also addressed, which makes this article a very comprehensive approach to the study of this rare, however dangerous disease.

All in all, even though it was not possible in this special series to cover all the possible model systems that recapitulate human ophthalmic pathologies, I believe we have put together an interesting and useful collection of protocols, together with an updated description of the related disease, that could be used in research laboratories to address the mechanisms behind

such pathologies, to lead the improvement in our diagnostic, prognostic and therapeutic knowledge.

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