

SUMMARY

Age-related maculopathy (ARM) is a severe threat to the visual ability of people over 65 years of age. ARM involves the central part of the retina where visual acuity is highest. In the late stages of ARM, called age-related macular degeneration (AMD), photoreceptor cells gradually disappear. The disease may be complicated by new vessels growing beneath the retina, called 'wet' or neovascular AMD. The causes and pathogenesis of this eye disease are not clear yet and unraveling slowly, thanks to many studies on this subject. Knowledge about these issues could eventually lead to therapies, and probably even more important, to strategies that prevent the disease from occurring. The purpose of this thesis was to study several of many molecular changes that occur during the development of ARM.

In the **first part (Chapters 1 - 3)** the clinical, microscopical and molecular characteristics of ARM are described and theories of possible mechanisms responsible for the development of ARM are discussed. Questions about the role of cell death during the aging process of the retina are outlined. The major part includes the role of angiogenic factors in the development of neovascular, or 'wet', AMD, in which abnormal vessels grow through the barriers of normal anatomy, towards the retina to form a choroidal neovascularization (CNV). Many so-called growth factors that play a role in CNV are identified up to now, but others need to be investigated.

With advancing age, the thickness of the retina decreases. Little is known about the way cells disappear during this process. In the **second part** of this thesis (**Chapter 4**) one way of cell death is studied, that is apoptosis, which can be viewed as a cell suicide program, present in all cells of the body. In this study, apoptosis was studied in the macula of rhesus monkeys of different ages. It was found that apoptotic cells were present at all ages, with an increase in the oldest monkey eyes, while the thickness of the retinal outer nuclear layer decreased with increasing age. The apoptosis-modulating proteins Bcl-x, Fas and Fas-ligand were expressed equally at all ages. These findings indicate that apoptosis in the primate macula occurs at all ages at similar rate, possibly increasing in the oldest age group, and may account for the decreasing thickness of the primate macula with age.

Dysfunctional retinal pigment epithelium (RPE) appears to play a central role in ARM, in combination with other factors eventually leading to neovascular AMD. The role of Fas and its natural ligand Fas-ligand (FasL) has been acknowledged in the process of angiogenesis. Fas and FasL induce apoptosis in T-lymphocytes but are also expressed on non-lymphoidal tissue. In the eye Fas-FasL interactions appear to be an important mechanism for the maintenance of immune privilege by inducing apoptosis

of invading lymphocytes. Recently, FasL expressed on RPE cells has been suggested to inhibit the growth and development of subretinal neovascularization. In **Chapter 5** a study is described in which FasL expression was investigated in the aging RPE and in early and late stages of ARM. FasL expression in RPE was not related to age or to the presence of early ARM. Furthermore, FasL expression in RPE was similar in subretinal and sub-RPE CNV. Thus, it appears to be unlikely that FasL expressed on RPE controls the extension of CNV from sub-RPE to subretinal.

In the **third part** of this thesis, several growth factors are studied that could be involved in the pathogenesis of neovascular AMD. In **Chapters 6 and 7** the Insulin-like Growth Factor family is investigated. IGF-I is a peptide that stimulates growth and differentiation of almost all cell types. The effects of IGF-I are regulated by the binding to six IGF-binding proteins (IGFBPs). Most of these IGFBPs have additional actions that are independent of IGF-I binding, including stimulation of cell growth and induction of apoptotic cell death. IGF-I is known to participate in each step of neovascularization. Therefore, the presence of IGF-I, its receptor (IGF-IR), and IGFBP-1 to -6 was examined in eyes with neovascular AMD at protein level and at mRNA level, which is an indication of the protein production in a cell. IGF-IR, little IGF-I, and most of the IGFBPs were shown in various cell types of CNV, both at protein and mRNA level. These results may point towards a role of this growth factor family in the pathogenesis of neovascular AMD. The functional role of the various IGF family members in AMD needs to be established.

It is becoming clear that a balance between stimulating and inhibiting growth factors regulates the growth of ocular neovascularization. Somatostatin reduces newly formed vessels by inhibiting the growth hormone/insulin-like growth factor axis and also has a direct anti-proliferative effect on various cell types involved in angiogenesis. In **Chapter 8** is demonstrated that most early-formed CNV in eyes of patients with AMD express sst_{2A} , which is a receptor for somatostatin. The sst_{2A} receptor binds potential anti-angiogenic somatostatin-analogues like octreotide. Therefore, somatostatin analogues may be an effective therapy in early stages of neovascular AMD.

In **Chapter 9** an experimental treatment for neovascular AMD is discussed. Radiotherapy has recently been employed to treat patients with neovascular macular degeneration in order to prevent severe visual loss. In this study the histopathological findings are described of a patient with neovascular AMD in both eyes, who was treated with low-dose radiotherapy 3 years before he died. No radiation-related histopathologic effect could be demonstrated following radiation therapy in this patient.

In **Chapter 10** the findings of the studies described are considered in view of the current knowledge. Problems encountered are discussed and a theoretical model on the pathogenesis of ARM is outlined. Suggestions for future research are made and the characteristics for the ideal animal model for research on ARM are discussed.