Research Projects

ANGIOPOIETIN-LIKE 4 IN DIABETES

Role of ANGPTL4 in the development of macular edema and loss of vision in patients with diabetic retinopathy.

The goal of this project is to elucidate the role of Angiopoietin-like 4 (ANGPTL4) in the promotion of pathological neovascularization and macular edema in diabetic retinopathy, the number one cause of blindness in the American working population. Our investigations use genetic, molecular biology and cell biology approaches as well as mouse models of ischemic and diabetic retinopathies.

Diabetes is one of the major contributors to disease and premature mortality worldwide. In the United States, more than 100 million adults live with diabetes or prediabetes, according to a report released in 2021 by the Centers for Disease Control and Prevention (CDC). If sugar levels are not managed appropriately, different chronic complications of the disease may occur as a result of oxidative stress, vascular damage, and immune dysfunction.

Diabetic retinopathy (DR) is a type of ischemic retinopathy and one of the most common complications in patients suffering from diabetes. Abnormal sugar levels initiate microvascular degeneration in the retina, leading to hypoxic stress and subsequent retinal neovascularization. Initial vascular damage causes leakage of fluids and blood, which produce edema and swelling of the macula, causing loss of central vision. This early stage of DR in which diabetic macular edema (DME) may be observed is called non-proliferative diabetic retinopathy (NPDR). Microvascular damage also causes hypoxic stress and leads to abnormal hypoxia-induced angiogenesis. These fragile new vessels often bleed into the vitreous, causing the development of floaters and even blockage of all vision. This more advanced stage of diabetic eye disease when the retina starts growing new blood vessels is called proliferative diabetic retinopathy (PDR). Unfortunately, the current treatments for NPDR and PDR (laser and anti-VEGF drugs) are only successful in half of the treated patients, underscoring the urgency to identify safe and effective therapeutic targets for the treatment of these patients.

We recently found that Angiopoietin-like 4 (ANGPTL4), a member of the family of Angiopoietin-like proteins (ANGPTLs) and a homolog of Angiopoietin 1 (ANGPT1) and 2 (ANGPT2), promotes the destabilization of the integrity of the vascular barrier, causing vascular hyperpermeability. These observations prompted us to investigate whether this factor could be involved in the induction of macular edema that is observed in DR. For this, we started a collaboration with Dr. Akrit Sodhi, from the Wilmer Eye Institute (Johns Hopkins Hospital, Baltimore, MD), who investigates the molecular pathogenesis of HIF-dependent retinal neovascularization. These collaborative efforts have yielded very exciting findings that suggest that ANGPTL4 may serve as an alternative therapy for patients with DR. Interestingly, we have found elevated ANGPTL4 levels in DR patients. We observed that ANGPTL4 is highly upregulated in hypoxic (glial) Müller cells and that this secretion causes the loss of vascular integrity of surrounding retinal capillaries. Our data shows that ANGPTL4 causes destabilization of endothelial cell-endothelial cell junctions through the rapid activation of RhoA and the contraction of

actomyosin fibers. An ANGPTL4-induced delayed activation of Rac1 and Cdc42 helps restore vascular integrity. Intraretinal co-injections of ANGPTL4 and VEGF in mice demonstrate that these factors cooperate in the induction of vascular hyperpermeability. Furthermore, we found that ANGPTL4 causes migration of retinal endothelial cells, promoting angiogenesis in the development of PDR, the most advanced stage of the disease.

We are currently studying the signal transduction pathways that are activated by ANGPTL4 to cause retinal vascular leakage and neovascularization. Our current investigations are focused on the identification of membrane receptors and intracellular effectors that are involved in the vasoactive functions of this important cytokine.

ANGIOPOIETIN-LIKE 4 IN HEAD AND NECK CANCER

Role of ANGPTL4 in the lymphatic and hematogenous dissemination and cisplatin chemoresistance of cancer cells in head and neck squamous cell carcinoma.

The goal of this project is to elucidate the role of ANGPTL4 in the metastatic dissemination and cisplatin chemoresistance of head and neck squamous cell carcinoma, the tumor that accounts for 4% of all cancers in the United States and the 6th most common cancer worldwide. Our investigations use genetic, molecular biology and cell biology approaches as well as mouse models of head and neck cancer and tumor metastasis.

Head and neck squamous cell carcinoma (HNSCC) affects about 65,000 new individuals in the US each year. The etiology of HNSCC appears to be multifactorial and strongly related to diet, lifestyle (tobacco alone or in betel, and alcohol use) or viral infection (HPV). HNSCC prognosis highly depends on the stage of the tumor, the presence of distant metastasis originated from the circulation of cancer cells through vascular blood vessels (*hematogenous* spread) or lymphatic blood vessels (*lymphatic* spread), and the health status of the patient. In HNSCC, metastasis through the lymphatics and lymph node colonization are common and represent a major factor for HNSCC poor prognosis. In addition, cisplatin chemotherapy, the gold standard treatment for advanced HNSCC tumors, often faces loss of responsiveness because of the onset of drug chemoresistance, increasing the possibility of relapse.

ANGPTL4 mRNA and protein were found to be increased in HNSCC biopsies and to correlate with cancer cell migration and presence of lymph node metastasis. Interestingly, we observed that ANGPTL4 is upregulated both in malignant and in premalignant HNSCC-derived cell lines. Our research interest is to elucidate the molecular mechanisms by which ANGPTL4 promotes HNSCC dissemination. We are exploring the hypothesis that upregulation and secretion of ANGPTL4 in HNSCC not only increases the migratory capacity of cancer cells but also their proliferative potential, through autocrine and paracrine pathways. We also have evidence that suggest the role of ANGPTL4 in the development of platinum-based chemoresistance in HNSCC, through the promotion of DNA damage response (DDR) and RAD51-dependent homologous recombination (HR). For this purpose, we are using a series of premalignant and malignant head and neck cancer cell lines, HNSCC biopsies and patient-derived tumor organoids.

Head and neck cancer presents an important therapeutic challenge that has proven relatively resistant to attempts to improve patient outcomes over the past several decades. We believe that our investigations on the role of ANGPTL4 in HNSCC are highly relevant as they may help identify novel molecular targets for the therapeutic management of patients suffering from this devastating disease.