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Date: February 28, 2020

Review of the PhD thesis of Katherine Falkowski,

"Analyzing the proteolytic network interactions of tissue kallikreins and matrix metalloproteinases "

Background

The extracellular matrix (ECM), essentially made up of collagens, elastin, laminins, aggrecans and other proteoglycans is a reservoir of latent and active growth factors and modulatory enzymes, especially proteases. The delicate homeostasis of this connective tissue relies on a balanced proteolytic network comprising activating and dampening (inhibitory) players.

Katherine Falkowski's thesis work is focused on the regulation of this proteolytic web in the context of periodontal disease, chronic inflammation, cancer and wound healing. It has been known since the seminal work of Jerome Gross and Charles Lapiere (PNAS, 1962) that matrix metalloproteinases (MMPs) are key players in extracellular matrix remodeling. Originally however, MMP function was attributed as predominantly destructive, for example in diseases like osteoarthritis or cancer metastasis. However, it is now generally accepted that MMPs are also involved in many beneficial physiological functions such as growth factor and chemokine activation and thus play important roles in organ development and tissue differentiation.

The proteolytic cascade leading to activation of MMPs has been studied for many years, but there are some long standing enigmas. One is the situation in periodontal disease, where infection by bacteria such as *Porphyromonas gingivalis* contribute proteolytic enzymes termed gingipains. There were indications and hypotheses suggesting that gingipains might activate

host proteases and thereby might manipulate the physiological homeostasis of the periodontal extracellular matrix in a destructive way.

Aims and Results

Besides host MMPs and bacterial gingipains, there are also other candidate proteases present in the context of periodontal disease, such as host serine proteases of the kallikrein type (tissue kallikreins, KLKs).

To address the question whether host proMMPs could be activated by sequential proteolysis through kallikreins and gingipains, Katherine Falkowski developed and used an elegant *in vitro* assay system in order to overcome the limited accessibility of sufficient amounts of proMMP molecules.

She used a carrier protein, originally discovered in *Porphyromonas*, which was known to be resistant to proteolysis. This carrier was recombinantly expressed including amino-terminal elongations of His-tagged peptides containing the activation-cleavage sites of proMMPs. This CleavEx-approach was applied to generate a library of all 23 human proMMP sequences and screened with tissue kallikreins for cleavage.

Active KLK14 cleaved ten MMP profragments, which were verified by mass spectrometry and Edman sequencing. Importantly, membrane bound proMMP sequences such as MMP14 (MT1-MMP) were cleaved most efficiently. MMP14 has been known to be the master initiator of MMP activation cascades. This was confirmed by analysis of the full-length proMT-MMPs, especially proMT1-MMP (proMMP14).

Furthermore Katherine Falkowski could demonstrate that this activation cascade is significantly enhanced by microbial gingipains, which effectively activate prokallikreins and destroy counteracting inhibitory proteins.

Thereby, gingipains promote the pathogenicity by boosting inflammation and possibly tumor growth and metastasis, thus linking periodontal disease and oral cancer.

Resume

The results presented by Katherine Falkowski provide fascinating new insights into the delicately regulated proteolytic web within the periodontal extracellular matrix. Parts of this work have already been published and the core data – not yet published - will have high impact in the field of extracellular matrix proteolysis.

The thesis work is excellently structured and written. The figures are of highest quality and intuitively comprehensible.

I shall recommend to the high faculty to highly honor Katherine Falkowski for this PhD thesis work. Based on the high quality and thoughtful interpretation of results of her thesis, I support awarding her a PhD attributed with the remark “with distinction” and request the Biological Sciences Council of Jagiellonian University to admit Katherina Falkowski to further stages of the doctoral dissertation procedure.

February 28, 2020

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