RECENT HIGH-IMPACT PAPERS FROM RADBOUDUMC RESEARCHERS

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With over 3,000 publications each year, scientific research is a cornerstone of the Radboud university medical center [1]. In this section, recent high-impact papers – published by researchers from the Radboudumc – will be discussed.

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Investigating protein-protein interactions with a new proximity labelling technique

When investigating the function of a protein, it can be incredibly helpful to know which other proteins interact with your protein of interest. Information on what other kinds of proteins are found in its neighbourhood can, for example, be a starting point for studying the function of a protein of interest. Proximity biotinylating is a powerful technique that allows identification of protein-protein interactions *in vivo*. In this technique, a proximity biotinylating enzyme, e.g., BioID, is fused to the protein of interest using a CRISPR-Cas9 mediated knock-in or a plasmid-based transfection. When biotin is added to cells containing this construct, proteins proximal to the protein of interest are biotinylated by the proximity biotinylating enzyme. The biotinylated proteins can then be isolated and subsequently analysed using mass spectrometry. However, a major drawback of this technique is that it can only be performed in cells that can be genetically engineered. To overcome this limitation, Santos-Barriopedro *et al*. published an article in *Nature communications* (impact factor = 14.9) in which they developed a modified proximity biotinylating technique that relies on a recombinant enzyme called ProtA-Turbo [2]. ProtA-Turbo consists of a proximity biotinylating enzyme fused to Protein A. Protein A allows for localisation of the recombinant enzyme to an antibody targeting the protein of interest. The authors show the functionality of ProtA-Turbo working together with multiple antibodies against both proteins and histone modifications. In the demonstration of their methods, they identified FLYWCH1 as an unreported proximal protein for the H3K9me3 histone modification ($p<0.05$). This technique is applicable in any cell type and thus allows for the identification of proteins located closely to any protein or histone modification for which an antibody is available.

When is gallbladder removal beneficial?

In about 5% of people with gallstones, complications can arise, such as intense pain in the abdomen, nausea, and vomiting. A common treatment for these symptomatic patients is a cholecystectomy, which entails the surgical removal of the gallbladder. However, many patients who undergo this surgery still experience pain afterwards, including some patients where pain persists and does not subside. To improve decision-making in treatment for patients with gallstone associated pain symptoms, Latenstein *et al*. analysed data from two multicentre prospective trials to identify predictors of pain reduction after cholecystectomy, which was reported in *JAMA Surgery* (impact factor = 14.8) [3]. Patients were evaluated for pain symptoms six months after their cholecystectomy. The study identified seven factors that could be used as predictors of clinically relevant pain reduction. Clinically relevant pain reduction was found with higher frequency in patients who were older, had no previous abdominal surgery, had a high pain score, and had pain radiating towards the back. Patients with clinically relevant pain reduction were also shown to report pain reduction with simple analgesics, nausea during pain attacks, and no heartburn (C statistic = 0.8, 95% CI 0.74-0.84). In the future, surgeons could use these predictors to assess the likelihood of pain reduction after cholecystectomy in their patients. In this way, the results from this study might help contribute to a reduction in cholecystectomies without pain relief and thus prevent patients from having redundant surgeries.

Dysregulation of autophagy impairs neuronal development

Koolen-de Vries syndrome (KdVS) is a rare neurodevelopmental disorder that has been linked to heterozygous loss of function mutations in the *KANSL-1* gene. This gene is likely important in the regulation of autophagy, a process in which the cell degrades its own components. A study by Linda *et al*. published in *Autophagy* (impact factor = 16.0) aimed to better understand how *KANSL-1* mutations affect neuronal function in KdVS patients by using iNeurons, which are neurons derived from human induced pluripotent stem cells (iPSCs) [4]. iNeurons from KdVS patients were found to have less functional synapses than healthy controls and showed a pattern of neuronal signalling associated with immature networks. As *KANSL-1* had previously been shown to be relevant for autophagy regulation, dysregulation of autophagy was further pursued as the potential cause of KdVS neuronal dysfunction. When using a marker for autophagosomes and immunofluorescence microscopy, it was found that KdVS neurons had a much higher amount of autophagosomes. To explain why this accumulation of autophagosomes occurred, the authors investigated differentially expressed genes in KdVS and found that expression of SOD1, an important antioxidant enzyme, was decreased. Due to a deficiency in SOD1, reactive oxygen species (ROS) levels were much higher in KdVS cells, which in turn induced the accumulation of autophagosomes. To further confirm this theory, iNeurons were treated with aponycin, a drug that inhibits ROS production. Upon treatment with aponycin, KdVS iNeurons had lowered ROS levels and reduced autophagosome accumulation ($p<0.01$, $p<0.005$). Furthermore, when iPSCs were treated with aponycin during differentiation towards iNeurons, functional synapse numbers and neuronal signalling resembled that of healthy control cells. Taken together, these findings indicate that the increased ROS and autophagosome accumulation found in KdVS patient neurons interfere with neuronal development. Therefore, antioxidants like aponycin, which rescued the KdVS phenotype in iNeurons, could be an interesting possibility for treatment of KdVS patients. Moreover, mutations in *KANSL-1* have been linked to Parkinson's disease in a genome wide association study, meaning that the findings from this paper might help contribute to increased understanding not only of KdVS, but potentially also of Parkinson's disease.

References

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