Hitting virus infections where it hurts

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Credit: AI-generated image (disclaimer)

Virus infection starts at the cell surface with interactions between viral and host proteins that bind together. Researchers are working on ways to lock out the virus or prevent it from reproducing in the cell by targeting these proteins.

The dynamic relationship between viruses and host cells they are infecting is dominated by carbohydrates called glycans and glycan-

binding proteins (GBPs). These complex interactions control entry of virus into the cell, virus replication in the cell and recognition of the virus by the host's immune system followed by possible neutralisation and elimination of the infection.

Glycan analysis is very demanding from a technical point of view and to date has not been addressed apart from one study. Partners from that study have now joined forces in the EU-funded HTP-GLYCOMET project. Together with the coordinating institution UNIRI, and another three partners with complementary experience in glycans and their binder molecules, they made major progress in understanding key processes involved in immunity and viral infections.

A massive span of applications for glycans

Areas of expertise within the consortium covered a wide range of themes. This included production of specialised monolithic chromatographic tools for high-throughput (HTP) fractionation of complex biological fluids, purification of proteins from body fluids and membrane proteins, HTP glycomic analysis using chromatography as well as multiplexed capillary gel electrophoresis topped off with expertise in the field of viral immunology.

Research has generated more than 25 peer-reviewed papers covering topics as wide-ranging as influenza A to glycosylation patterns in patients undergoing image-guided tumour ablation.

High-throughput is the key

HTP-GLYCOMET researchers developed a high-throughput method for investigation of protein glycosylation. "We then applied it for analysis of these posttranslational modifications (PTMs) of serum and plasma

membrane proteins," outlines Prof. Djuro Josic, coordinator of HTP-GLYCOMET.

Working on influenza and murine (mouse) cytomegalovirus, the team successfully produced a range of monoclonal antibodies and established HTP protocols for antibody purification. Subsequent isolation of low-abundance serum and membrane proteins and optimisation of HTP liquid-chromatography and mass spectrometry has resulted in identification and characterisation of glycoproteins and their corresponding glycan structures.

Project research was not without its challenges and demanded intuitive experience from the scientific partners. Isolation of membrane proteins and characterisation of their glycan parts sometimes proved difficult. The solution was optimisation of both the solubilisation and enzymatic deglycosylation of highly glycosylated and hydrophobic proteins.

Loss of activity of the monoclonal antibodies after their immobilisation was another hitch. Development of new monoclonal antibodies together with optimisation of the immobilisation chemistry was the painstaking answer to this problem.

A bright future for glycoprotein research

Discussing the work for the future, Prof. Josic outlines, "We will continue with high-throughput isolation of serum and plasma membrane glycoproteins and analysis of changes of their glycosylation during pathological changes." As for breaking into the market of analysis of glycoproteins, "The new protein immobilisation technology on the surface of monolithic supports and newly developed ELISA plates with 96 monolithic disks will be commercially used for high-throughput analysis of serum glycoproteins." The disks, with immobilised protein L are geared for HTP isolation of different immunoglobulins, mainly IgG,

IgM and IgA.

The HTP-GLYCOMET project has made progress in an area previously left unresearched due to technical difficulties working with glycoproteins. The work has established a firm knowledge foundation for glycan maps during infection and the continuing development of a dedicated glycan-assignment database.

Findings could be applied in biomedical areas to develop new drugs or vaccines, determine response to surgery or therapy as well as development of innovative personalised therapies.

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