Analysis of the site-specific *N*-glycosylation of the HeLa cell lysate

Simon Sugár, András Ács, Ágnes Gömöry, Gábor Tóth, Károly Vékey, László Drahos, Lilla Turiák

MS Proteomics Research Group, Hungarian Academy of Sciences, Research Centre for Natural Sciences, Budapest, Hungary

Objectives:

I. Analysis of the site-specific *N*-glycosylation of HeLa proteins

II. Assessment of the degree of contamination originating from the culture media

Conclusions:

I. First in-depth study of site-specific HeLa *N*-glycosylation – enhanced reliability of results and novel information on HeLa *N*-glycosylation

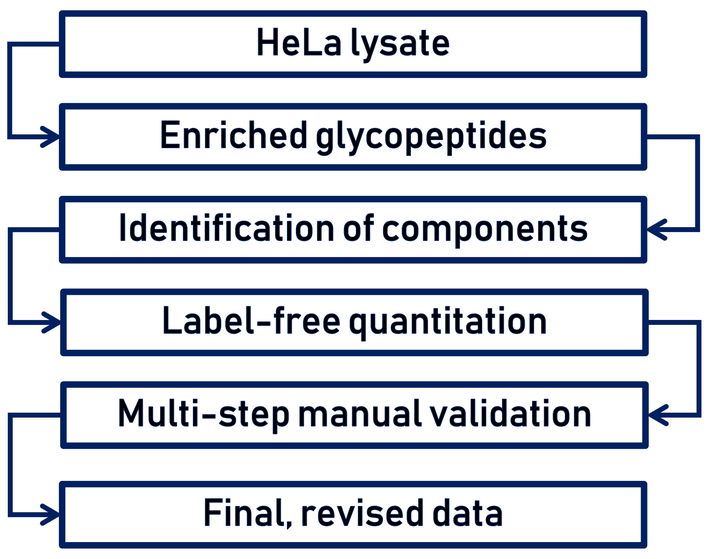
II. High degree of contamination from culture media causes significant bias – HeLa glycosylation should only be studied in a site-specific manner

Introduction:

- HeLa cell lines:
  derived from cervical cancer tissue, commonly used in cancer research and as an MS standard, commercially available in lysate form
- HeLa *N*-glycosylation:
  information on the *N*-glycosylation of HeLa proteins is lacking altough glycosylation carries potentially important biological information
- Site-specific glycopeptide analysis:
  glycopeptides are analyzed in intact form opposed to analysing the glycan and peptide moieties separately – more valuable information but more challenging due to lower abundance as opposed to non-glycosylated peptides

Workflow:

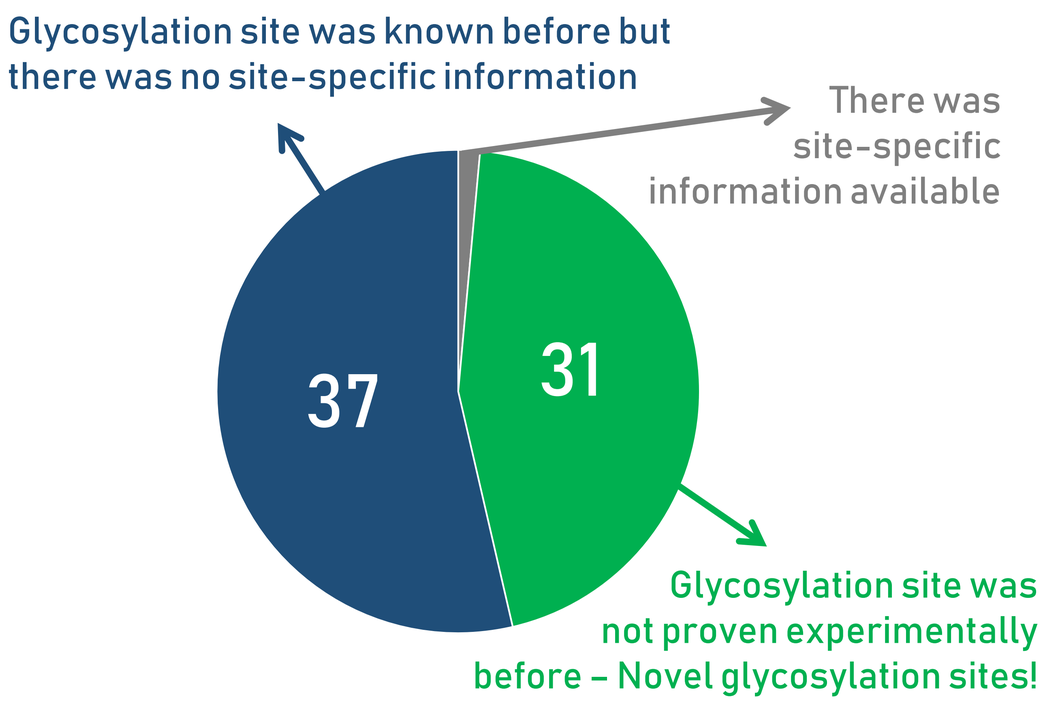
- Complex workflow: glycopeptide enrichment followed by identification of components via HPLC-DDA-MS/MS and label free quantitation using HPLC-MS data using our in-house built GlycoPattern software
- The focus was not on maximizing the quantity of identified glycopeptides but on enchanced reliability
- Introduction of a multi-step manual data validation process, which involved the evaluation of specific high and low energy MS/MS spectra



HeLa glycosylation:

- Over 40 glycoproteins identified with 69 glycosylation sites, 28 different glycan structures overall 178 different glycopeptides with high confidence

The identified glycosylation sites:



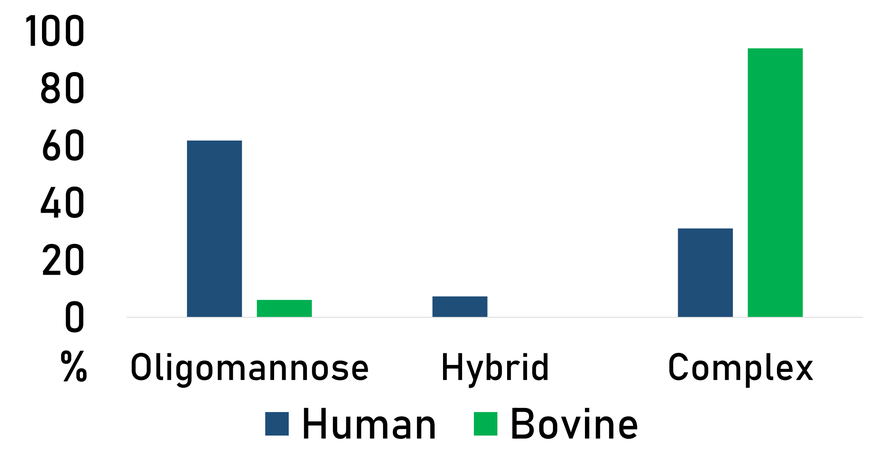
Bovine contamination:

- Commercially available HeLa cell digest (Thermo Scientific, Waltham, USA) contained high amounts of bovine protein contaminants likely from the FBS culture media
- Data suggested that previous studies on HeLa may have been biased especially released glycan analyes, where the origin of the glycan structures can not be determined

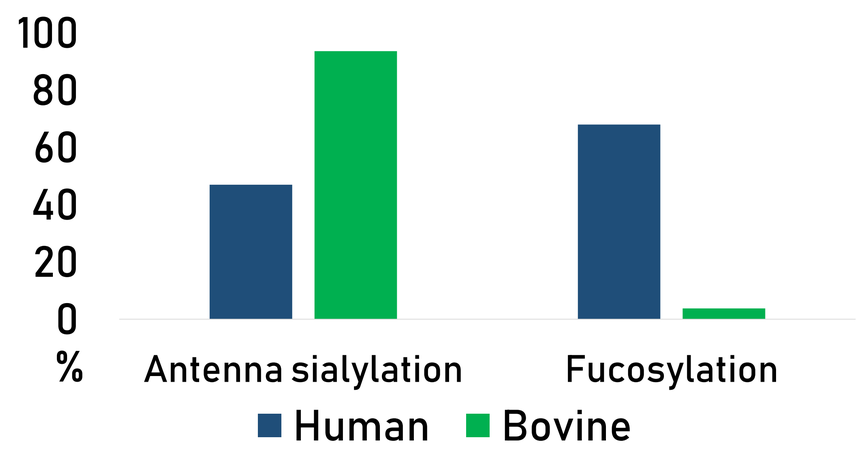
Effects and degree of contamination:

- Approximately the same amount of bovine and human glycopeptides identified in the sample
- The glycosylation pattern of the bovine proteins is very different from the human proteins

Partition of major glycan types by organisms:

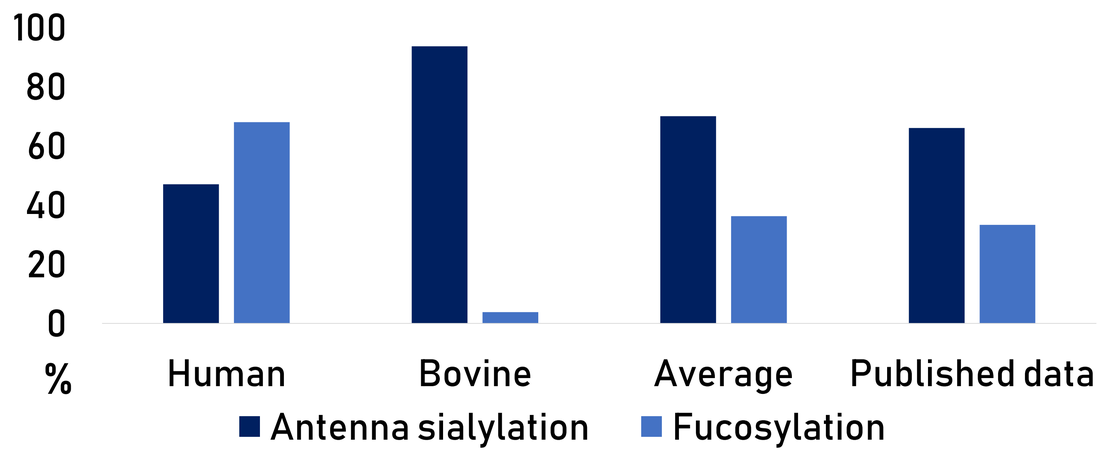


Sialylation and fucosylation by organisms:



Comparison of results with published data:

- Comparison of our experimental data on the *N*-glycosylation pattern of HeLa proteins with a previously published released glycan analysis of HeLa* indicate significant bias
- Results clearly show, that due to the high amount of bovine protein contaminants the sialylation and fucosylation levels published are similar to our averaged human & bovine data



REFERENCES: *: Gao, Wenjie, et al. "A facile method for cellular N-glycomic profiling by matrix-assisted laser desorption/ionization mass spectrometry." RSC Advances 7.57 (2017): 35687-35693.

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