PHOSPHOPROTEOMICS ANALYSES IN CELIAC DISEASE

Gaetana Paolella

Ivana Caputo

Principal investigators
Gaetana Paolella
Ivana Caputo

Other components of the research group
Stefania Martucciello
Silvia Sposito
Carla Esposito

Background

Celiac disease (CD) is a complex intestinal inflammatory condition caused by a specific response to peptides derived from ingested gliadin in genetically predisposed individuals. Gut lumen is reached not only by immunogenic peptides, but also by another class of gliadin peptides (of which p31-43 is the prototype), able to trigger a local innate immune and proliferative response. It enters cells, probably by a direct interaction with cell membranes (Paolella et al., Cell Biol Intern 2018) and induce a wide range of biological effects on cells. Some studies have highlighted the ability of p31-43 to act as a growth factor, as it induces ERK phosphorylation, cytoskeleton rearrangements and proliferation (Barone et al. Gut 2007; Caputo et al., BBA 2010). P31-43 also contributes to the inflammatory response, caused by an increased production of ROS (Luciani et al. Gut 2010). Finally, a constitutive alteration in signalling and proliferation rate in celiac cells has been demonstrated, both at the

site of inflammation and far from the intestine (Nanayakkara et al. PLoS One 2013). This important observation may partially explain why the damaging effects of p31-43 are specific for celiac cells.

Since proteins phosphorylation represents a key mechanism for the regulation of several biological processes, we aim to characterize the constitutive signalling alteration found in celiac patients, by analyzing the phosphoproteome composition of their cells with respect to cells from healthy controls. We also aim to investigate how phosphoproteome is modified in the presence of p31-43. By means of this analysis, we will able to highlight different responses occurring in normal and celiac cells.

Main achievements

We previously contributed to delineate some growth factor-like effects of p31-43 in a model of epithelial intestinal cells (Caco-2 cells) (Caputo et al. Plos One 2012). Indeed, we found that p31-43 rapidly mobilized calcium ions from intracellular stores (both mitochondria and ER), thus inducing an ER-stress response. Calcium mobilization was also sufficient to induce the activation of the cross-linking activity of intracellular type 2 transglutaminase, an enzyme exerting a crucial role in CD triggering, and itself involved in other signalling pathways. In these years we also developed the technical capacity to identify phosphorylated proteins in a biological sample in the context of CD pathogenesis. In particular, we studied phosphoproteome modifications induced by anti-type 2 transglutaminase antibodies (Paolella et al. Plos One 2013), which represent another class of molecules acting as growth factors in CD.

Future perspectives

We will characterize the constitutive signalling alteration found in celiac patients by analyzing the phosphoproteome composition of their cells with respect to cells from healthy controls. As study model, we will use cultures of fibroblasts from skin explants, but also Caco-2 cells, which are useful to set experimental conditions. We will also investigate how phosphoproteome is modified, both qualitatively and quantitatively, in the presence of p31-43. The experimental strategy will involve the employment of a two-dimensional electrophoresis approach to separate phosphoproteins, possibly combined with a phospho-specific enrichment method, and mass spectrometry (MS) analysis to identify proteins showing changes in phosphorylation level, and to identify phosphorylation sites which are involved. MS-data will be validated and supported by bioinformatics analysis of functional categories and pathways.

Publications

1. *Caputo I, Secondo A, Lipretti M, Paolella G, Auricchio S, Barone MV, Esposito C. Gliadin peptides induce tissue transglutaminase activation and ER-stress through Ca2+ mobilization in Caco-2 cells. PLos One 2012; 7(9):e45209 https://doi.org/10.137/journal.pone.0045209.*
2. *Caputo I, Lepretti M, Secondo A, Martucciello S, Paolella G, Sblattero D, Barone MV, Esposito C. Amino Acids 44(1):251-60. https://doi.org/10.1007/s00726-011-1120-y.*
3. *Paolella G, Caputo I, Marabotti A, Lepretti M, Salzano AM, Scaloni A, Vitale M, Zambrano N, Sblattero D, Esposito C. Celiac anti-type 2 transglutaminase antibodies induce phosphoproteome modification in intestinal epithelial Caco-2 cells. PLoS One 2013; 8(12):e84403.*
4. *Vangone A, Abdel-Azeim S, Caputo I, Sblattero D, Di Niro R, Cavallo L, Oliva R. Structural basis for the recognition in an idiotype-anti-idiotype antibody complex related to celiac disease. PLoS One 2014; 30; 9(7):e102839. https://doi.org/10.1371/journal.pone.0102839.*
5. *Paolella G, Lepretti M, Barone MV, Nanayakkara M, Di Zenzo M, Sblattero D, Auricchio S, Esposito C, Caputo I. Celiac anti-type 2 transglutaminase antibodies induce differential effects in fibroblasts from celiac disease patients and from healthy subjects. Amino Acid 2017; 49(3):541-550. https://doi.org/10.1007/s00726-016-2307-z.*
6. *Martucciello S, Paolella G, Esposito C, Lepretti M, Caputo I. Anti-type 2 transglutaminase antibodies as modulators of type 2 transglutaminase functions: a possible pathological role in celiac disease. Cell Mol Life Sci 2018; 75(22):4107-4124. doi: 10.1007/s00018-018-2902-0.*
7. *Paolella G, Lepretti M, Martucciello S, Nanayakkara M, Auricchio S, Esposito C, Barone MV, Caputo I. The toxic alpha-gliadin peptide 31-43 enters cells without a surface membrane receptor. Cell biol Intern 2018; 42(1):112-120. https://doi.org/10.1002/cbin.10874.*

External collaborations

- *Secondo A, Department of Neuroscience, Reproductive and Odontostomatological Sciences, University Federico II, Naples, Italy*
- *Sblattero D, Department of Life Sciences, University of Trieste, Italy*
- *Oliva R, Department of Sciences and Technologies, University Parthenope, Naples, Italy*
- *Scaloni A, ISPAAM, CNR, Naples, Italy*