David, Leonor

Abstract

Mucins and mucin glycosylation in *Helicobacter pylori* infection

Leonor David, Raquel Almeida, Celso A. Reis, Luis Filipe Santos Silva
IPATIMUP and Medical Faculty of the University of Porto, Rua Dr. Roberto Frias s/n, 4200 Porto, Portugal. Mail: ldavid@ipatimup.pt

The relevance of mucins and mucin glycosylation for *Helicobacter pylori* (HP) colonization of the gastric mucosa stems from *in vivo* and experimental observations. Both the protein and the carbohydrate components of mucin glycoproteins, and also the carbohydrate moiety of glycolipids, have been shown to play a role in HP adhesion. MUC1 and MUC5AC gastric mucins have been shown to interact with HP and carbohydrate structures Le^b, H type 1, and sialyl-Le^x have been identified as ligands for HP adhesins.

Our group has been involved in the study of mucins and mucin glycosylation in normal and pathological conditions and the findings that are relevant for HP colonization will be presented.

In the normal gastric mucosa and in chronic atrophic gastritis without intestinal metaplasia (IM) the expression of mucins/carbohydrates in the foveolar epithelium, where HP establishes its niche, consists of MUC1, MUC5AC and type 1 Lewis structures depending on the Lewis (FUT3) and Secretor (FUT2) genes. The relevance of these structures has therefore been postulated and demonstrated in experimental and in vivo conditions. Supportive of their relevance is also that colonization of duodenal mucosa by HP is related to gastric metaplasia with expression of MUC5AC. In IM, on the other hand, there is clearance of HP from most metaplastic glands. We have shown that clearance of HP from IM occurs when gastric mucins are lost and MUC2 intestinal mucin is aberrantly expressed ("complete" IM). Persistence of HP is observed in the cases of "incomplete" IM that maintain expression of MUC5AC and show undetectable expression of MUC2. This observation suggests that either MUC5AC is essential for HP colonization or that MUC2 is deleterious for HP binding. The recent identification of a major mechanism for transactivation of the MUC2 gene by the intestinal homeobox gene CDX2 will allow the generation of in vitro models where the relevance of MUC2 in HP binding can be assayed. Other structures, mainly glycan structures, may also be involved in the clearing of HP from IM. One such structure is the sialyl-Tn that is also aberrantly expressed in IM, mainly co-localizing with MUC2 in goblet cells. Recently the ST6GalNAc-I was identified as the major STn synthase and gastric carcinoma cell lines stably transfected with ST6GalNAc-I will be used to clarify the relevance of STn for binding of HP. The observation that the α1,4-GlcNAc, expressed in gastric glands, has an antimicrobial activity in the normal mucosa led us to search for its abnormal/increased expression in IM. We could not demonstrate any relevance for HP clearance based on α1,4-GlcNAc inappropriate expression in IM.

The relevance of Lewis/Secretor status in HP adhesion *in vivo* is controversial. A study of gastric biopsies evaluated for Lewis/Secretor phenotypes/genotypes did not disclose associations with HP infection, in contrast to what was observed for Secretor status in Norwalk virus adhesion, suggesting that, if relevant, it may depend upon the HP BabA status.

Finally, we will present two model systems under investigation: 1. Evaluation of the role of MUC1 mucin expression/glycosylation on HP adhesion; 2. Identification of glycosyltransferases induced by co-culture of gastric cells with HP using a Glyco-gene Chip array.

References from the group:

1.Teixeira A et al: **Expression of mucins (MUC1, MUC2, MUC5AC and MUC6) and of type 1 Lewis antigens in cases with and without *Helicobacter pylori* colonization in metaplastic glands of the human stomach.** J Pathol 197: 37-43, 2002.

2. Serpa J et al: **Lewis enzyme (a1-3/4 fucosyltransferase) polymorphisms do not explain the Lewis phenotype in the gastric mucosa of a portuguese population.** J Hum Genet 48: 183-189, 2003.

3. Mesquita P et al: **Human MUC2 mucin gene is transcriptionally regulated by CDX homeodomain proteins in gastrointestinal carcinoma cell lines.** J Biol Chem 278: 51549-51556, 2003.

4. Marcos NT et al: **Role of the human ST6GalNAc-I and ST6GalNAc-II in the synthesis of the cancer-associated sialyl-Tn antigen.** Cancer Res 64:7050-7057, 2004

5. Serpa J et al: **Two new FUT2 (fucosyltransferase 2 gene) missense polymorphisms, 739G-->A and 839T-->C, are partly responsible for non-secretor status in a Caucasian population from Northern Portugal.** Biochem J 383:469-474, 2004

6.Santos-Silva F et al: **Thomsen-Friedenreich antigen expression in gastric carcinomas is associated with MUC1 mucin VNTR polymorphism.** Glycobiology 15:511-517, 2005.

7. Ferreira B, et al: **Terminal α1,4-linked N-acetylglucosamine in Helicobacter pylori-associated intestinal metaplasia of the human stomach and gastric carcinoma cell lines.** J Histochem Cytochem 2006 (in press).

Curriculum Vitae

Leonor David

Leonor David is Pathologist and Full Professor of Pathology at the Medical Faculty of Porto, did her training as a PhD student and pos-doc at the Radium Hospital in Norway and at the University of Copenhagen, participated in the organizing committee of several international meetings on cancer and mucin/mucin glycosylation, acted as supervisor of 7 PhD students in Medicine and Human Biology, published 95 papers in journals with peer-review, has written 5 book chapters, was member of the Council of the European Association for Cancer Research, acts as Reviewer for several Journals in the fields of cancer, gastroenterology and mucin glycobiology, is member of the Editorial Board of "Critical Reviews in Oncogenesis", is a group leader of a research group in "Oncogenesis" at IPATIMUP.

Research interests: Mucins and mucin glycosylation in gastric carcinogenesis.

Main Publications:

David L, Leitão D, Sobrinho-Simões M, Bennett E, White T, Mandel U, Dabelsteen E, Clausen H: **Biosynthetic basis of incompatible histo-blood group A antigen expression : Anti-A transferase antibodies reactive with gastric cancer tissue of type O individuals.** Cancer Res 53:5494-5500, 1993

Reis CA, David L, Nielsen P, Clausen H, Mirgorodskaya K, Roepstorff P, Sobrinho-Simões M: **Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibody.** Int J Cancer 74: 112-121, 1997.

Carvalho F, Seruca R, David L, Amorim A, Seixas M, Bennet E, Clausen H, Sobrinho-Simões M: **MUC1 gene polymorphism and gastric cancer - an epidemiologic study**. Glycoconjugate J 14: 107-111, 1997.

Reis CA, David L, Correa P, Carneiro F, Garcia E, de Bolós C, Mandel U, Clausen H, Sobrinho-Simões M: **Intestinal metaplasia of human stomach displays distinct patterns of expression of mucins MUC1, MUC2, MUC5AC and MUC6.** Cancer Res 59: 1003-1007, 1999.

Silva F, carvalho F, Peixoto A, Seixas M, Almeida R, Carneiro F, Mesquita P, Figueiredo C, Nogueira C, Swallow DM, Amorim A, David L: **MUC1 gene polymorphism in the gastric carcinogenesis pathway.** Eur J Hum Genet 9: 548-552, 2001.

Teixeira A, David L, Reis CA, Costa J, Sobrinho-Simões M: **Expression of mucins (MUC1, MUC2, MUC5AC and MUC6) and of type 1 Lewis antigens in cases with and without *Helicobacter pylori* colonization in metaplastic glands of the human stomach.** J Pathol 197: 37-43, 2002.

Almeida R, Silva E, Silva F, Silberg DG, Wang J, de Bolós C, David L: **Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas.** J. Pathol 199: 36-40, 2003.

Mesquita P, Peixoto AJ, Seruca R, Hanski C, Almeida R, Silva F, Reis C, David L: **Role of site-specific promoter hypomethylation in aberrant MUC2 mucin expression in mucinous gastric carcinomas.** Cancer Letters 28: 129-136, 2003.

Serpa J, Almeida R, Oliveira C, Silva F, Silva E, Reis C, Le Pendu J, Oliveira G, Cunha-Ribeiro LM, David L: **Lewis enzyme (a1-3/4 fucosyltransferase) polymorphisms do not explain the Lewis phenotype in the gastric mucosa of a portuguese population.** J Hum Genet 48: 183-189, 2003.

Silva F, Carvalho F, Peixoto A, Teixeira A, Almeida R, Reis C, Bravo LE, Realpe L, Correa P, David L: **MUC1 polymorphism confers increased risk for intestinal metaplasia in a Colombian population with chronic gastritis.** Eur J Hum Genet 11:380-384, 2003.

Mesquita P, Jonckheere N, Almeida R, Ducourouble M-P, Serpa J, Silva E, Pigny P, Silva F, Reis CA, Silberg D, Van Seuningen I, David L: **Human MUC2 mucin gene is transcriptionally regulated by CDX homeodomain proteins in gastrointestinal carcinoma cell lines.** J Biol Chem 278: 51549-51556, 2003.

Serpa J, Mendes N, Reis CA, Santos-Silva F, Almeida R, Le Pendu J, David L: **Two new FUT2 missense polymorphisms, 739G>A and 839T>C, are partly responsible for non-secretor status in a Caucasian population from Northern Portugal**. Biochem J 383: 469-474, 2004.

Santos-Silva F, Fonseca A, Caffrey T, Carvalho F, Mesquita P, Reis C, Almeida R, David L, Hollingsworth MA: **Thomsen-Friedenreich antigen expression in gastric carcinomas is associated with MUC1 mucin VNTR polymorphism.** Glycobiology 15:511-517, 2005.

Almeida R, Almeida J, Shoshkes M, Mendes N, Mesquita P, Silva E, Van seuningen I, Reis CA, Santos-Silva F, David L: **OCT-1 is over-expressed in intestinal metaplasia and intestinal gastric carcinomas and bind to, but does not transactivate, CDX2 in gastric cells**. J Pathol 207: 396-401, 2005.