Gremlin1 and chronic pancreatitis: a new clinical target and biomarker?

Derek P. Brazil¹

Published online: 28 August 2015
© Springer-Verlag Berlin Heidelberg 2015

Chronic pancreatitis (CP) is a progressive inflammatory condition triggered by the loss of exocrine pancreatic acini and fibrosis or scarring of pancreas tissue [1]. The exocrine pancreas comprises about 90 % of total pancreas and releases digestive enzymes such as chymotrypsin, amylase and lipase in response to a meal. CP develops in 16 % of patients presenting with acute pancreatitis, a number which increases to 38 % upon second admission. The most common symptom of CP is abdominal pain, which can be both intermittent and persistent. Furthermore, 95 % of CP patients present with alcoholic or idiopathic disease [1]; however, excessive consumption of alcohol alone may not be the cause of CP. Additional causes of CP include gallstones, exposure to volatile hydrocarbons, viral infection and genetic mutations [2]. There are various genetic forms of CP including gain-of-function mutations in genes such as *Prss1* which results in a form of trypsin that is resistant to degradation and can lead to an autosomal-dominant hereditary form of CP [1]. In contrast, loss-of-function mutations in genes such as *Spink1* and *Cfr*, which is involved in the inactivation of pancreatic trypsin, are also associated with CP. The overall survival rate for CP patients is only 50 % at 20–25 years from diagnosis [3], and CP patients also have an increased risk of developing pancreatic cancer [4].

Current treatments for CP involve pain management with paracetamol and non-steroid anti-inflammatory drugs as first line, followed by mild opioids such as tramadol. An important

clinical feature of CP is steatorrhea, caused by reduced absorption of fat in the intestine. Supplementation of pancreatic enzymes such as lipase, together with H₂ histamine antagonists, is used to reduce this malabsorption of lipids [1]. Micro-nutrient therapy containing antioxidants such as Antox[®] (containing methyl and thiol moieties, as well as methionine and vitamin C) is now being tested, as it has been shown to control the pain and attacks associated with CP [1].

The pathogenesis of CP is believed to be associated with inappropriate pancreatic enzyme activation and clearance from the acinar cells. Increased pancreatic Ca²⁺ levels as a result of excessive consumption of alcohol or other causes is common in CP, with calcifying protein deposits implicated in the blockage of the acinar ducts, leading to digestive enzyme accumulation and subsequent inflammation. Other evidence suggests that noxious agents such as alcohol are directly toxic to the acinar cells and may also trigger the activation of pancreatic stellate cells [5]. An important element of the pathogenesis of CP is fibrosis, which can occur via sustained activation of pancreatic stellate cells that migrate to sites of tissue damage and secrete excess extracellular matrix proteins, causing fibrosis or scarring [6]. Factors that drive this fibrotic response include lipid peroxidation products released by acinar cells and TGF-β1 release by reactive oxygen species (ROS)-activated mast cells that infiltrate the pancreas as part of the inflammatory response to CP [1]. Progression of fibrosis in CP is associated with the loss of acinar and islet cells, leading to both exocrine and endocrine pancreas dysfunction. There are currently no effective therapies to prevent or reverse CP [7]. Given the insidious nature of CP, together with the increased mortality rate and pancreas cancer risk, improved treatment options for reversing the progressive fibrosis associated with this disease are urgently needed. To this end, new molecular targets that are involved in the onset and progression of fibrosis associated with CP need to be identified.

✉ Derek P. Brazil
d.brazil@qub.ac.uk

¹ Centre for Experimental Medicine, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7AE, Northern Ireland, UK

Gremlin1 is a secreted member of the cysteine knot DAN family of glycoproteins [8]. Gremlin1 binds to and antagonises bone morphogenetic proteins (BMPs) in the extracellular matrix, preventing BMP receptor activation and downstream signalling [8]. Gremlin1 is involved in physiological processes such as limb and digit formation and nephrogenesis [9]. Increased Gremlin1 expression has been identified in fibrosis associated with kidney, lung, heart and liver disease [10, 11]. Targeting Gremlin1 by gene deletion or shRNA knockdown reduced the severity of kidney disease in mouse models of diabetic nephropathy (DN) [12, 13]. A genetic variant of Gremlin1 is associated with increased risk of diabetic nephropathy [14]. A novel role for Gremlin1 as a pro-angiogenic ligand of the VEGFR2 receptor has also been identified [15]. Relevant to this finding, several groups have identified increased Gremlin1 levels in a range of cancers, including colon, pituitary, malignant mesothelioma, cervical and pancreas cancers [16, 17]. Importantly, levels of Gremlin1 have been shown to be elevated in human biopsies from patients with DN [11], pulmonary arterial hypertension (PAH) [10], non-ischaemic heart failure [18] and liver fibrosis [19]. In this issue of J Mol Med, Staloch and colleagues have identified a new role for Gremlin1 as a novel biomarker and potential therapeutic target in CP [20]. The molecular targets of Gremlin1, BMPs, have been shown to antagonise the pro-fibrotic action of TGF- β 1 in fibrosis [20]. BMP-2 inhibits TGF- β 1 activation of pancreatic stellate cell and extracellular matrix secretion [21]. During CP progression, BMP activity is reduced and TGF- β 1 activity is increased, causing fibrosis and CP progression [20]. The current report from Staloch and colleagues suggests that upregulation of Gremlin1 represents a mechanism by which anti-fibrotic BMP action is inhibited during CP, allowing the pro-fibrotic action of TGF- β 1 to dominate.

CP can be induced in mice via intraperitoneal injection of the cholecystokinin analogue cerulein [20]. Marked collagen deposition was evident in pancreatic sections of cerulein-injected mice. Increased levels of pancreatic TGF- β 1 was detected after 1 week, together with increased Gremlin1 expression which peaked at week 3 post-CP induction. Mice haploinsufficient for Gremlin1 (*gremlin1*^{+/-}) displayed attenuated pancreatic collagen deposition in response to cerulein at week 4 post-injection. Isolated mouse pancreatic stellate cells (mPSCs) showed increased Gremlin1 expression in response to TGF- β 1, and recombinant Gremlin1 was able to block BMP-2-mediated inhibition of TGF- β 1-induced collagen expression. Importantly, increased levels of Gremlin1 were detected in the pancreata of CP patients, confirming the relevance of these findings to human disease.

So, what are the clinical implications of these data? CP is a complex disease that involves both an inflammatory component and a fibrotic injury. Given the paucity of treatment options to delay or reverse fibrosis in CP, the BMP/Gremlin1

axis may represent a novel pathway that can be targeted in CP. The balance of signalling factors that regulate pancreatic stellate cells is perturbed during CP, with increased Gremlin1 expression appearing to dampen anti-fibrotic BMP signalling, thus exacerbating TGF- β 1-mediated fibrosis (summarised in Fig. 1). Treatment strategies that inhibit TGF- β 1 would, *prima facie*, have the potential to improve patient outcome in CP. However, targeting of TGF- β 1 is limited by the potential for serious side-effects due to the pleiotropic nature of TGF- β 1 action in the body. Focus has therefore turned to downstream targets of TGF- β 1 such as CTGF and Gremlin1 as potential targets in fibrosis. Given the wealth of data implicating Gremlin1 in fibrosis of the kidney, lung, heart, liver and now pancreas, it would seem that strategies to inhibit Gremlin1 would have obvious clinical potential. This view was supported by a report from a Novartis group who demonstrated that antibody-mediated targeting of Gremlin1 reduced vascular remodelling and improved the outcome of mice with PAH [22]. This paper provides an important proof-of-concept for those researchers interested in developing pharmacological tools aimed at targeting Gremlin1 action in fibrosis in CP patients. Advances in this field have been achieved by the identification of molecules such as tilorone and Thr123 which activate BMP-7 signalling and reduce lung and kidney fibrosis [23, 24]. Pharmacological inhibition of Gremlin1 would also be of interest to colleagues working in the cancer field, as elevated Gremlin1 levels have been detected in numerous cancers. Indeed, Gremlin1 has been suggested as a pro-angiogenic factor capable of activating VEGFR2, which may contribute to its role in tumour development [25]. An important role for Gremlin1 resides in its maintenance of

Fig. 1 Summary of Gremlin1-mediated fibrosis in chronic pancreatitis. TGF- β 1 signalling to pancreatic stellate cells (PSCs) drives fibrosis via expression of extracellular matrix proteins such as fibronectin and collagen, and also increases α -smooth muscle actin expression (α -SMA). This pro-fibrotic signalling is counteracted by BMP-2 signalling, which in turn is inhibited by increased Gremlin1 expression from PSCs triggered by TGF- β 1 and other factors. Thus, increased Gremlin1 is both a driver and marker of fibrosis during chronic pancreatitis

cancer stem cell (CSC) pluripotency, which has been implicated in colon cancer and glioma initiation and development [26, 27]. Thus, pharmacological targeting of Gremlin1 in this scenario would drive CSCs towards a more differentiated tumour cell type that would be more susceptible to chemotherapy and radiotherapy agents [27]. Given that CP progresses to pancreas cancer in approximately 7 % of patients, this suggests that Gremlin1 may have overlapping roles in both fibrosis and cancer. Some outstanding questions remain to be answered, such as, is Gremlin1-mediated inhibition of BMPs the sole pro-fibrotic mechanism at play during CP? Is the increase in Gremlin1 expression in CP part of an acute repair process in the pancreas that becomes dysregulated in chronic disease? Also, since Gremlin1 is a secreted protein, can we detect elevated Gremlin1 levels in the circulation of patients with CP or other forms of fibrosis? In the connective tissue disorder Loeys-Dietz syndrome, Gremlin1 plasma levels of 858 ng/ml were detected, compared to 349 ng/ml in healthy donors [28]. It would be interesting to explore the potential of Gremlin1 as a circulating marker of fibrosis in CP and other conditions such as DN and PAH. New high-affinity Gremlin1 antibodies would greatly enhance our ability to detect Gremlin1 in patient plasma or serum samples.

In conclusion, CP can now be added to the growing list of fibrotic disorders in which Gremlin1 is implicated. Staloch and colleagues shed new light on the pathophysiology of CP and identify the Gremlin1/BMP signalling axis as a novel therapeutic target for the treatment of CP. Future work will determine if targeting of Gremlin1 can provide benefit in delaying or reversing the progression of CP in patients.

Acknowledgments Work in the author's laboratory is supported by Diabetes UK, BBSRC, Northern Ireland Kidney Research Fund and DEL NI. The author thanks Dr. Lana McClements for the assistance in proof-reading the manuscript.

References

1. Braganza JM, Lee SH, McCloy RF, McMahon MJ (2011) Chronic pancreatitis. *Lancet* 377:1184–1197
2. Dufour MC, Adamson MD (2003) The epidemiology of alcohol-induced pancreatitis. *Pancreas* 27:286–290
3. Ammann RW (2006) Diagnosis and management of chronic pancreatitis: current knowledge. *Swiss Med Wkly* 136:166–174
4. Layer P, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMaggio EP (1994) The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology* 107:1481–1487
5. Witt H, Apte MV, Keim V, Wilson JS (2007) Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology* 132:1557–1573
6. Omary MB, Lugea A, Lowe AW, Pandol SJ (2007) The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J Clin Invest* 117:50–59
7. Warshaw AL, Banks PA, Fernandez-Del Castillo C (1998) AGA technical review: treatment of pain in chronic pancreatitis. *Gastroenterology* 115:765–776
8. Brazil DP, Church RH, Suras S, Godson C, Martin F (2015) BMP signalling: agony and antagonism in the family. *Trends Cell Biol* 25: 249–264
9. Khokha MK, Hsu D, Brunet LJ, Dionne MS, Harland RM (2003) Gremlin is the BMP antagonist required for maintenance of Shh and Fgf signals during limb patterning. *Nat Genet* 34:303–307
10. Cahill E, Costello CM, Rowan SC, Harkin S, Howell K, Leonard MO, Southwood M, Cummins EP, Fitzpatrick SF, Taylor CT et al (2012) Gremlin plays a key role in the pathogenesis of pulmonary hypertension. *Circulation* 125:920–930
11. Walsh DW, Roxburgh SA, McGettigan P, Berthier CC, Higgins DG, Kretzler M, Cohen CD, Mezzano S, Brazil DP, Martin F (2008) Co-regulation of Gremlin and Notch signalling in diabetic nephropathy. *Biochim Biophys Acta* 1782:10–21
12. Roxburgh SA, Kattla JJ, Curran SP, O'Meara YM, Pollock CA, Goldschmeding R, Godson C, Martin F, Brazil DP (2009) Allelic depletion of gremlin attenuates diabetic kidney disease. *Diabetes* 58: 1641–1650
13. Zhang Q, Shi Y, Wada J, Malakauskas SM, Liu M, Ren Y, Du C, Duan H, Li Y, Li Y et al (2010) In vivo delivery of Gremlin siRNA plasmid reveals therapeutic potential against diabetic nephropathy by recovering bone morphogenetic protein-7. *PLoS One* 5, e11709. doi:10.1371/journal.pone.0011709
14. McKnight AJ, Patterson CC, Pettigrew KA, Savage DA, Kilner J, Murphy M, Sadler D, Maxwell AP, Warren UKGoKiDSG (2010) A GREM1 gene variant associates with diabetic nephropathy. *J Am Soc Nephrol: JASN* 21:773–781
15. Mitola S, Ravelli C, Moroni E, Salvi V, Leali D, Ballmer-Hofer K, Zammataro L, Presta M (2010) Gremlin is a novel agonist of the major proangiogenic receptor VEGFR2. *Blood* 116:3677–3680
16. Karagiannis GS, Musrap N, Saraon P, Treacy A, Schaeffer DF, Kirsch R, Riddell RH, Diamandis EP (2015) Bone morphogenetic protein antagonist gremlin-1 regulates colon cancer progression. *Biol Chem* 396:163–183
17. Namkoong H, Shin SM, Kim HK, Ha SA, Cho GW, Hur SY, Kim TE, Kim JW (2006) The bone morphogenetic protein antagonist gremlin 1 is overexpressed in human cancers and interacts with YWHAH protein. *BMC Cancer* 6:74
18. Mueller KA, Tavlaki E, Schneider M, Jorbenadze R, Geisler T, Kandolf R, Gawaz M, Mueller II, Zuern CS (2013) Gremlin-1 identifies fibrosis and predicts adverse outcome in patients with heart failure undergoing endomyocardial biopsy. *J Card Fail* 19: 678–684
19. Boers W, Aarass S, Linthorst C, Pinzani M, Elferink RO, Bosma P (2006) Transcriptional profiling reveals novel markers of liver fibrogenesis: gremlin and insulin-like growth factor-binding proteins. *J Biol Chem* 281:16289–16295
20. Staloch D, Gao X, Liu K, Xu M, Feng X, Aronson JF, Falzon M, Greeley GH, Rastellini C, Chao C et al (2015) Gremlin is a key pro-fibrogenic factor in chronic pancreatitis. *J Mol Med*. doi:10.1007/s00109-015-1308-9
21. Gao X, Cao Y, Yang W, Duan C, Aronson JF, Rastellini C, Chao C, Hellmich MR, Ko TC (2013) BMP2 inhibits TGF-beta-induced pancreatic stellate cell activation and extracellular matrix formation. *Am J Physiol Gastrointest Liver Physiol* 304:G804–G813
22. Ciucian L, Sheppard K, Dong L, Sutton D, Duggan N, Hussey M, Simmons J, Morrell NW, Jarai G, Edwards M et al (2013) Treatment with anti-gremlin 1 antibody ameliorates chronic hypoxia/SU5416-induced pulmonary arterial hypertension in mice. *Am J Pathol* 183:1461–1473
23. Lepparanta O, Tikkanen JM, Bernal MM, Koli K, Myllarniemi M (2013) Bone morphogenetic protein-inducer tilorone identified

- by high-throughput screening is antifibrotic in vivo. *Am J Respir Cell Mol Biol* 48:448–455
24. Sugimoto H, LeBleu VS, Bosukonda D, Keck P, Taduri G, Bechtel W, Okada H, Carlson W Jr, Bey P, Rusckowski M et al (2012) Activin-like kinase 3 is important for kidney regeneration and reversal of fibrosis. *Nat Med* 18:396–404
 25. Koketsu K, Yoshida D, Kim K, Ishii Y, Tahara S, Teramoto A, Morita A (2015) Gremlin, a bone morphogenetic protein antagonist, is a crucial angiogenic factor in pituitary adenoma. *Int J Endocrinol* 2015:834137
 26. Davis H, Irshad S, Bansal M, Rafferty H, Boitsova T, Bardella C, Jaeger E, Lewis A, Freeman-Mills L, Giner FC et al (2015) Aberrant epithelial GREM1 expression initiates colonic tumorigenesis from cells outside the stem cell niche. *Nat Med* 21:62–70
 27. Yan K, Wu Q, Yan DH, Lee CH, Rahim N, Tritschler I, DeVecchio J, Kalady MF, Hjelmeland AB, Rich JN (2014) Glioma cancer stem cells secrete Gremlin1 to promote their maintenance within the tumor hierarchy. *Genes Dev* 28:1085–1100
 28. Wellbrock J, Sheikhzadeh S, Oliveira-Ferrer L, Stamm H, Hillebrand M, Keyser B, Klokow M, Vohwinkel G, Bonk V, Otto B et al (2014) Overexpression of Gremlin-1 in patients with Loeys-Dietz syndrome: implications on pathophysiology and early disease detection. *PLoS One* 9, e104742. doi:[10.1371/journal.pone.0104742](https://doi.org/10.1371/journal.pone.0104742)