CCN3: the-pain-killer inside me

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Received: 11 March 2012 / Accepted: 12 March 2012 / Published online: 30 March 2012
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Abstract There is increasing evidence that metalloproteinases are involved in neuropathic pain [Dev et al., Expert Opin Investig Drugs 19:455–468 2010] Hence, the identification of molecules that can regulate MMP9 and MMP2 is warranted. In a recent publication, Kular et al. (2012) claim that CCN3 functions to decrease inflammatory pain via the regulation of two metalloproteinases, MMP2 and MMP9, in response to experimentally-induced inflammation. Their conclusion is based on the following observations : i) the expression of CCN3 was reduced following induction of pain by subcutaneous injection of complete Freund's adjuvant in rat's paw, ii) an inhibition of MMP9 decreased CFA-associated mechanical allodynia, iii) inhibition of CCN3 expression by siRNA led to an upregulation of MMP2 in the dorsal horn of the spinal cord (DHSC) and MMP9 in the dorsal root ganglia (DRG), iv) a partial effect of CCN3 on CFA-induced expression of MMP9 and MMP2 in DRG and DHSC following intrathecal injection of CCN3. Unfortunately, the conclusion of this study is weakened by the lack of experimental evidence showing a direct relationship between the expression of CCN3 and MMPs. Furthermore, several results contained in this manuscript only confirm data that were previously established by others. Owing to the wide range of activities which have been attributed to CCN3 (Perbal, Mol Pathol 54:57–79 2001, Brigstock, J Endocrinol 178:169–175 2003, Perbal, Lancet 363 (9402):62–64 2004, Perbal, Cell Commun Signal 4:6 2006, Holbourn et al. Trends Biochem Sci. 33:461–473 2008, Leask and Abraham, J Cell Sci 119:4803–4810 2006, Jun and Lau, Nat Rev Drug Discov 10:945–963

2011, Rachfal and Brigstock, Vitam Horm 70:69–103 2005), the mechanisms underlying the potential role of CCN3 in the expression of these MMPs in the context of inflammatory pain must be thoroughly studied before a meaningful conclusion can be reached. Indeed, Kular et al. description of variations in CCN3, MMP9 and MMP2 levels occurring simultaneously is not sufficient to draw a functional relationship between these three proteins. It should be noted that the expression of CCN3 was already reported to repress MMP9 (Benini et al., Oncogene 24:4349–4361 2005, Fukunaga-Kalabis et al., Oncogene 27:2552–2560 2008) and the roles of CCN3 in inflammatory processes has been extensively documented in the past few years (Bleau et al., Front Biosci 10:998–1009 2005, Lin et al., J Biol Chem 280:8229–8237 2005, Perbal, Cell Commun Signal 4:6 2006, Hughes et al., Diabetologia 50:1089–1098 2007, Lin et al., J Cell Commun Signal 4:141–153 2010, Pasmant et al., J Neuropathol Exp Neurol 69:60–69 2010, Shimoyama et al., Thromb Vasc Biol 30:675–682 2010, Lemaire et al., J Invest Dermatol 130:2517 2010, Chen and Lau, J Cell Commun Signal 4:63–69 2010, Le Dréau et al., Glia 58:1510–1521 2010, Rittié et al. J Cell Commun Signal 5:69–80 2011, Janune et al., J Cell Commun Signal 5:167–171 2011). In addition, the expression of CCN3 in the neurons of dorsal root ganglia and dorsal horn of the spinal horn in rat and human has also been documented (Su et al., C R Acad Sci III 321:883–892 1998, Mol Pathol 54:184–191 2001, Kocialkowski et al., Anat Embryol (Berl) 203:417–427 2001). Implication of CCN3 in cognitive functions (Su et al., Sheng Li Xue Bao 52:290–294 2000) and the possible involvement of CCN3 in the regulation of pain was already suggested almost a decade ago (Perbal, Expert Rev Mol Diagn 3:597–604 2003, Perbal et al., Mol Pathol 56:80–85 2003) with the demonstration of cell-specific effects of CCN3 on intracellular calcium stores and inhibition of anionic channels by CCN3 (Li et al., Mol Pathol

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55:250–261 2002, Lombet et al., *Cell Commun Signal* 1:1 2003, Perbal, *Expert Rev Mol Diagn* 3:597–604 2003, Perbal et al., *Mol Pathol* 56:80–85 2003). Aside from these general aspects, and in the light of the potential participation of CCN3 in the whole process of pain sensing, the reader would have appreciated the discussion in this manuscript not being essentially a flat summary of the data presented, but a more thorough discussion of the possible role for CCN3 in the regulation of MMPs and its significance in the context of the wide biological functions of CCN3.

Keywords CCN proteins · CCN2 · CCN3 · Pain · Pain killer · Allodynia · Neuropathic pain · Metalloproteinases · MMP9 · MMP2 · Inflammation · Inflammatory pain · Neuroinflammation · Brain · Nervous system · Calcium · Calcium channel · Calcium signaling · Calcium inhibitors · Anionic channels · Neurotransmission · Treatment of pain · Nerve conduction

Scientifique perspective

An increasing body of evidence supports the idea that proteins of the extracellular matrix (ECM) are major players in the global control of intercellular communication and integration of environmental signals. Among them, the members of the CCN family of matricellular proteins constitute a new group of signaling regulators.

As previously suggested (Perbal 2001; 2004) the unique tetramodular structure of CCN proteins sets the stage for combinatorial events leading to the wide array of cell and environment specific biological functions that have been attributed to CCN proteins (Perbal 2001 and manuscript in preparation).

Each of the four constitutive modules of CCN proteins allows physical and functional interactions with large families of proteins and ligands that are involved in the control of various aspects of signaling.

Along this line, processes that control the temporal and local bioavailability of CCN partners are critical to confer on these proteins their wide range of specific functions and provide clues to better understand how proteins sharing so much identity may exhibit adverse effects (Perbal 2001 and manuscript in preparation). This situation may indeed explain apparently contradictory observations that are, in fact, due to the very nature of the multimolecular complexes in which CCN proteins are involved at a precise time in a precise location.

Hence, the unique structure of CCN proteins is likely to confer on these proteins a central role in physically networking signaling pathways that need to be activated or silenced in a coordinated fashion in response to a wide array of stimuli from birth to death (Perbal manuscript in preparation).

Amongst the many biological functions attributed to CCN proteins over the past decade, a role for CCN3 in biological functions of the nervous system was first suggested by the detection of CCN3 in kidney's neuronal soma (Chevalier et al. 1998). Interestingly the CCN3 protein was detected in neuronal processes, indicating that it was synthesized in neuron cell bodies and transported along axonal processes, an observation suggesting a possible role of CCN3 in nerve conduction.

A comprehensive analysis of CCN3 expression in the central nervous system (CNS) of human and rat were performed in collaboration with the laboratory of Pr. Cai (Chongqing, China), Pr. Schofield (Cambridge, UK) and Dr. Yeger (Toronto, Canada). Our published data (Su et al. 1998, 2001; Kocialkowski et al. 2001) established that expression of CCN3 followed a spatio-temporal regulation in the developing CNS. Neurons positive for CCN3 were first detected in the spinal cord, both in the ventral region at 16 weeks of gestation (G16W) and in the dorsal region at G38W. A positive staining for CCN3 was also detected at G16W in the principal nucleus of the inferior olive, the hypoglossal nucleus and the dorsal motor nucleus of vagus and at G28W in the nucleus of the spinal tract of the trigeminal and cuneate nucleus. At G38W the abducens nucleus of pons, the red nucleus and the substantia nigra of the midbrain, the ventral posterolateral and the medio-dorsal thalamic nucleus stained positive. A strong labeling was also detected in the striatum of the cerebrum and the cerebral cortex of the parietal lobe.

Therefore CCN3 is mainly expressed in somato-motor neurons in the lower central nervous system at early developmental stages and in the higher central nervous system at later stages.

In the late phase of first trimester development, CCN3 labeling of the floor plate becomes prominent and in the caudal (lumbar) portion of the spinal cord is accompanied by labeling of the cell bodies of neurons in the ventral horns. High levels of CCN3 signal are seen in the dorsal root ganglia (DRG) in all of the ages examined.

The first evidence that suggested a possible role for CCN3 in the treatment of pain, was obtained in the course of studies which established that CCN3 and CCN2 proteins participated in the regulation of calcium flux and mobilisation of internal stores (Li et al. 2002; Lombet et al. 2003; Perbal 2003).

Indeed, a wide array of observations point to a critical role of calcium signaling in the transmission of pain signals. For example, calcium channels are known to be key mediators of nociceptive signaling in the DRG (Altier and Zamponi 2004), and altered expression of sodium channels was proposed to account for decrease nociceptive thresholds to mechanical stimuli (Nishimura et al. 2010). More recently, blockade of voltage-gated Ca^{2+} channels on sensory nerves was shown

attenuates neurotransmitter release and membrane hyperexcitability associated with chronic pain states.

Critique

In their analysis of inflammatory pain, Kular et al. (2012) did not consider the aforementioned important physiological effects of CCN3. They only focused their attention on the observed variations in the levels of two metalloproteinases MMP9 and MMP2 upon manipulation of CCN3 expression levels.

The involvement of MMP9 and MMP2 in the development and maintenance of neuropathic pain has been extensively studied (Dev et al. 2010; Zhang et al. 2011) and the roles of CCN proteins in various inflammatory processes has been largely documented and remains under intense investigation (Bleau et al. 2005; Lin et al. 2005; Perbal 2006; Hughes et al. 2007; Lin et al. 2010; Pasmant et al. 2010; Shimoyama et al. 2010; Lemaire et al. 2010; Chen and Lau 2010; Le Dréau et al. 2010; Rittié et al. 2011; Janune et al. 2011).

In order to claim that CCN3 «attenuates inflammatory pain through regulation of matrix metalloproteinases -2 and -9» would first require establishing unequivocally that these two proteinases are indeed involved in inflammatory pain, and second demonstrating that CCN3 plays a role in the regulation of MMP2 and MMP9 expression in the effector cells.

Thus in the absence of a mechanistic approach, the data reported Kular et al. (2012) do not fulfill these important requirements.

Even though a qualitative link between MMP9 and CCN3 had previously been suggested in the case of Ewing's tumors (Benini et al. 2005), showing that ectopic variations of CCN3, in their model of CFA-induced pain, are accompanied by variations in the levels of these MMPs is not sufficient to establish functional relationships between these proteins and a role for CCN3 in the attenuation of pain.

As has been famously said : « When you remove the legs of a flea and order it to jump, its incapacity to do so does not imply that removing legs induces deafness »

A number of other claims within the manuscript would have required additional experimental support .

For example, data regarding the variation of CCN3 mRNA over time following CFA injection showed that the level of CCN3 dropped at day 15 in DRG. Curiously, the three flanking points (days 7, 21 and 60) showed the same levels as in the control. The authors then concluded that the expression of CCN3 is temporally regulated.

To arrive at this conclusion would require at least conducting a time-course type of experiment within a shorter window of time, around day 15 to document the kinetics of

CCN3 downregulation. This is critical to any kind of further conclusion.

In the same figure, the relative mRNA levels showed that after a drop at day 15 (about 60 %), CCN3 mRNA levels increased at days 21 and 60 reaching 40 % of the initial value in DHSC. Concomitantly, the amounts of CCN3 protein in DHSC, dropped gradually from day 7 (about 10 %) to day 60 (about 40 %). Upon examination these data do not appear to be in accordance with the author's statement that « NOV protein quantification... » is « consistent with the NOV RNA expression pattern in DHSC ». On this basis, although the data contained in this manuscript might be of interest in the frame of inflammatory pain, it would necessitate that the conclusions be extended, confirmed and sustained by unequivocal data.

A discussion addressing the biological significance of these observations, in the context of the pleiotropic CCN proteins functions, would have considerably strengthened the message of the manuscript.

Bearing in mind what has been presented here the conclusion drawn by the authors about the potential administration of CCN3 for the therapy of pain is challenged by critical aspects that have not yet been sufficiently addressed.

These are :

- 1) It is now well established that the expression of CCN3 and CCN2, two proteins showing antagonistic effects, is coordinately regulated in many tissues (Kawaki et al. 2008; Riser et al. 2009). Both are potent regulators of calcium channelling. Therefore is CCN2 expression involved in inflammatory pain and could it be a useful target ?
- 2) Since CCN3 is a strong inhibitor of anionic channels, sustained systemic injection of CCN3 might have dramatic consequences. This aspect is of considerable importance, as the aim, not to be forgotten, is to eliminate pain, and not the patient.

In summary, much more needs to be investigated regarding the role of CCN3 in the critical physiological aspects of pain and pain management. It is hoped that the insights provided in this critique will stimulate further research in the CCN field.

Acknowledgment The author is grateful to Dr. Herman Yeger for critical reading of this commentary.

References

- Altier C, Zamponi GW (2004) Targeting Ca²⁺ channels to treat pain: T-type versus N-type. *Trends Pharmacol Sci* 25(9):465–470
- Benini S, Perbal B, Zambelli D, Colombo MP, Manara MC, Serra M, Parenza M, Martinez V, Picci P, Scotlandi K (2005) In Ewing's sarcoma CCN3(NOV) inhibits proliferation while promoting

- migration and invasion of the same cell type. *Oncogene* 24 (27):4349–4361
- Bleau AM, Planque N, Perbal B (2005) CCN proteins and cancer: two to tango. *Front Biosci* 10:998–1009, Review
- Brigstock DR (2003) The CCN family: a new stimulus package. *J Endocrinol* 178(2):169–175, Review
- Chen CC, Lau LF (2010) Deadly liaisons: fatal attraction between CCN matricellular proteins and the tumor necrosis factor family of cytokines. *J Cell Commun Signal* 4(1):63–69, Epub 2009 Nov 7
- Chevalier G, Yeager H, Martinier C, Laurent M, Alami J, Schofield PN, Perbal B (1998) novH: differential expression in developing kidney and Wilm's tumors. *Am J Pathol* 152(6):1563–1575
- Dev R, Srivastava PK, Iyer JP, Dastidar SG, Ray A (2010) Therapeutic potential of matrix metalloproteinase inhibitors in neuropathic pain. *Expert Opin Investig Drugs* 19(4):455–468
- Fukunaga-Kalabis M, Martinez G, Telson SM, Liu ZJ, Balint K, Juhasz I, Elder DE, Perbal B, Herlyn M (2008) Downregulation of CCN3 expression as a potential mechanism for melanoma progression. *Oncogene* 27(18):2552–2560, Epub 2007 Oct 29
- Holbourn KP, Acharya KR, Perbal B (2008) The CCN family of proteins: structure-function relationships. *Trends Biochem Sci* 33(10):461–473, Epub 2008 Sep 11, Review
- Hughes JM, Kuiper EJ, Klaassen I, Canning P, Stitt AW, Van Bezu J, Schalkwijk CG, Van Noorden CJ, Schlingemann RO (2007) Advanced glycation end products cause increased CCN family and extracellular matrix gene expression in the diabetic rodent retina. *Diabetologia* 50(5):1089–1098, Epub 2007 Feb 28
- Janune D, Kubota S, Lazar N, Perbal B, Iida S, Takigawa M (2011) CCN3-mediated promotion of sulfated proteoglycan synthesis in rat chondrocytes from developing joint heads. *J Cell Commun Signal* 5(3):167–171, Epub 2011 May 11
- Jun JJ, Lau LF (2011) Taking aim at the extracellular matrix: CCN proteins as emerging therapeutic targets. *Nat Rev Drug Discov* 10(12):945–963. doi:10.1038/nrd3599, Review
- Kawaki H, Kubota S, Suzuki A, Lazar N, Yamada T, Matsumura T, Ohgawara T, Maeda T, Perbal B, Lyons KM, Takigawa M (2008) Cooperative regulation of chondrocyte differentiation by CCN2 and CCN3 shown by a comprehensive analysis of the CCN family proteins in cartilage. *J Bone Miner Res* 23(11):1751–1764
- Kocialkowski S, Yeager H, Kingdom J, Perbal B, Schofield PN (2001) Expression of the human NOV gene in first trimester fetal tissues. *Anat Embryol (Berl)* 203(6):417–427
- Kular L, Rivat C, Lelongt B, Calmel C, Laurent M, Pohl M, Kitabgi P, Melik-Parsadaniantz S, Martinier C (2012) NOV/CCN3 attenuates inflammatory pain through regulation of matrix metalloproteinases-2 and -9. *J Neuroinflammation* 9(1):36 [Epub ahead of print]
- Le Dréau G, Kular L, Nicot AB, Calmel C, Melik-Parsadaniantz S, Kitabgi P, Laurent M, Martinier C (2010) NOV/CCN3 upregulates CCL2 and CXCL1 expression in astrocytes through beta1 and beta5 integrins. *GLIA* 58(12):1510–1521
- Leask A, Abraham DJ (2006) All in the CCN family: essential matricellular signaling modulators emerge from the bunker. *J Cell Sci* 119(Pt 23):4803–4810, Review
- Lemaire R, Farina G, Bayle J, Dimarzio M, Pendergrass SA, Milano A, Perbal B, Whitfield ML, Lafyatis R (2010) Antagonistic effect of the matricellular signaling protein CCN3 on TGF-beta- and Wnt-mediated fibrillinogenesis in systemic sclerosis and Marfan syndrome. *J Invest Dermatol* 130(6):1514–1523, Epub 2010 Feb 25. Erratum in: *J Invest Dermatol*. 2010 Oct;130(10):2517. Perbal, Bernard [added]
- Li CL, Martinez V, He B, Lombet A, Perbal B (2002) A role for CCN3 (NOV) in calcium signalling. *Mol Pathol* 55(4):250–261
- Lin CG, Chen CC, Leu SJ, Grzeszkiewicz TM, Lau LF (2005) Integrin-dependent functions of the angiogenic inducer NOV (CCN3): implication in wound healing. *J Biol Chem* 280:8229–8237
- Lin Z, Natesan V, Shi H, Hamik A, Kawanami D, Hao C, Mahabaleshwar GH, Wang W, Jin ZG, Atkins GB, Firth SM, Rittié L, Perbal B, Jain MK (2010) A novel role of CCN3 in regulating endothelial inflammation. *J Cell Commun Signal* 4(3):141–153, Epub 2010 Aug 11
- Lombet A, Planque N, Bleau AM, Li CL, Perbal B (2003) CCN3 and calcium signaling. *Cell Commun Signal* 1(1):1
- Nishimura I, Thakor D, Lin A, Ruangsri S, Spigelman I (2010) Molecular strategies for therapeutic targeting of primary sensory neurons in chronic pain syndromes. In L Kruger, AR Light (Ed.), *Translational pain research: from mouse to man* (chapter 6). Boca Raton, FL, CRC Press
- Pasmant E, Ortonne N, Rittié L, Laurendeau I, Lévy P, Lazar V, Parfait B, Leroy K, Dessen P, Valeyrie-Allanore L, Perbal B, Wolkenstein P, Vidaud M, Vidaud D, Bièche I (2010) Differential expression of CCN1/CYR61, CCN3/NOV, CCN4/WISP1, and CCN5/WISP2 in neurofibromatosis type 1 tumorigenesis. *J Neuropathol Exp Neurol* 69(1):60–69
- Perbal B (2001) NOV (nephroblastoma overexpressed) and the CCN family of genes: structural and functional issues. *Mol Pathol* 54(2):57–79, Review
- Perbal B (2003) The CCN3 (NOV) cell growth regulator: a new tool for molecular medicine. *Expert Rev Mol Diagn* 3(5):597–604, Review
- Perbal B (2004) CCN proteins: multifunctional signalling regulators. *Lancet* 363(9402):62–64, Review
- Perbal B (2006) New insight into CCN3 interactions—nuclear CCN3: fact or fantasy? *Cell Commun Signal* 4:6
- Perbal B, Brigstock DR, Lau LF (2003) Report on the second international workshop on the CCN family of genes. *Mol Pathol* 56(2):80–85
- Rachfal AW, Brigstock DR (2005) Structural and functional properties of CCN proteins. *Vitam Horm* 70:69–103, Review
- Riser BL, Najmabadi F, Perbal B, Peterson DR, Rambow JA, Riser ML, Sukowski E, Yeager H, Riser SC (2009) CCN3 (NOV) is a negative regulator of CCN2 (CTGF) and a novel endogenous inhibitor of the fibrotic pathway in an in vitro model of renal disease. *Am J Pathol* 174(5):1725–1734, Epub 2009 Apr 9
- Rittié L, Perbal B, Castellot JJ Jr, Orringer JS, Voorhees JJ, Fisher GJ (2011) Spatial-temporal modulation of CCN proteins during wound healing in human skin in vivo. *J Cell Commun Signal* 5(1):69–80, Epub 2011 Jan 11
- Shimoyama T, Hiraoka S, Takemoto M, Koshizaka M, Tokuyama H, Tokuyama T, Watanabe A, Fujimoto M, Kawamura H, Sato S, Tsurutani Y, Saito Y, Perbal B, Koseki H, Yokote K (2010) CCN3 inhibits neointimal hyperplasia through modulation of smooth muscle cell growth and migration. *Arterioscler Thromb Vasc Biol* 30:675–682
- Su BY, Cai WQ, Zhang CG, Su HC, Perbal B (1998) A developmental study of novH gene expression in human central nervous system. *C R Acad Sci III* 321(11):883–892
- Su BY, Cai WQ, Xiong Y, Zhang CG, Perbal B (2000) Relationships between learning and memory and expression of nov gene of rats. *Sheng Li Xue Bao* 52(4):290–294
- Su BY, Cai WQ, Zhang CG, Martinez V, Lombet A, Perbal B (2001) The expression of ccn3 (nov) RNA and protein in the rat central nervous system is developmentally regulated. *Mol Pathol* 54(3):184–191
- Zhang H, Chang M, Hansen CN, Basso DM, Noble-Haeusslein LJ (2011) Role of matrix metalloproteinases and therapeutic benefits of their inhibition in spinal cord injury. *Neurotherapeutics* 8(2):206–220