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REVIEW

CCN3 – A key regulator of the hematopoietic compartment

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SUMMARY

CCN3, a founding member of the CCN family of growth regulators, was linked with hematology in 2003[1] when it was detected in human serum. CCN3 is expressed and secreted by hematopoietic progenitor cells in normal bone marrow. CCN3 acts through the core stem cell signalling pathways including Notch and Bone Morphogenic Protein, connecting CCN3 with the modulation of self-renewal and maturation of a number of cell lineages including hematopoietic, osteogenic and chondrogenic. CCN3 expression is disrupted in Chronic Myeloid Leukemia as a consequence of the *BCR–ABL* oncogene and allows the leukemic clone to evade growth regulation. In contrast, naïve cord blood progenitors undergo enhanced clonal expansion in response to CCN3. Altered CCN3 expression is associated with numerous solid tumors including glioblastoma, melanoma, adrenocortical tumours, prostate cancer and bone malignancies including osteosarcoma. Mature CCN3 protein has five distinct modules and truncated protein variants with altered function are found in many cancers. Regulation by CCN3 is therefore cell type and isoform specific. CCN3 has emerged as a key player in stem cell regulation, hematopoiesis and a crucial component within the bone marrow microenvironment.

Introduction

The CCN family

The CCN protein family was named using the initial letter of the first three founding members Cyr61 (CCN1), CTGF (CCN2) and Nephroblastoma overexpressed (CCN3). The family includes the WISP proteins 1–3 (Wnt induced secreted proteins) known as CCN4, CCN5 and CCN6. They share 60% amino acid homology and contain a signal peptide (SP) followed by 4 structural modules possessing 38 conserved cysteine residues.[2] The modules comprise an insulin-like growth factor binding protein domain (IGFBP), von Willebrand type C repeat (VWC), thrombospondin type I domain (TSP-1) and cysteine knot carboxyl terminal (CT) (Fig. 1). The growth regulatory CCN proteins are involved in a diverse array of processes including angiogenesis, tumorigenesis, chondrogenesis, apoptosis and hematopoiesis. CCN proteins are known to regulate cell proliferation, adhesion, migration, differentiation, apoptosis, growth arrest and extracellular matrix production. All CCN members have roles as extracellular, cytoplasmic and nuclear proteins that can regulate transcription by forming nuclear complexes even though devoid of a classic nuclear localization sequence. The four domain structure of the CCN protein family is consistent with their involvement in a wide spectrum of processes. To date, the majority of research has been conducted on the founding family members, CCN1-3 although reports are rapidly accumulating for the more recently identified members, CCN4-6.

CCN1 and CCN2 are important for vascular development; CCN1 is an essential regulator and acts in a pro-angiogenic manner through the integrins alpha (v) beta (3) and alpha (6) beta (1).[3–5] CCN1 is associated with sites of bone remodelling; osteoblasts produce CCN1 in response to a number of bone-related growth factors and CCN1 promotes collagen and cartilage deposition by chondrocytes. CCN2 plays roles in angiogenesis, chondrogenesis, endochondral ossification, fibrosis, atherosclerosis and wound healing.[5–7] CCN2 is produced in response to a variety of growth factors including transforming growth factor beta (TGFβ), basic fibroblastic growth factor (bFGF) and vascular endothelial growth factor (VEGF).[5] Both CCN1 and CCN2 regulate VEGF and bFGF expression thus depicting multi-tiers of regulation of angiogenesis.[5] CCN1 is associated with advanced disease in breast cancer, disease progression and poor prognosis[8,9] whereas CCN2 is linked with steroid dependent breast cancer.[8] CCN1 expression in neuroblastoma and prostate cancer negatively correlates with malignant phenotype and is associated with growth inhibition of non small cell lung cancer. CCN4-6 (WISP1-3), also have roles in breast cancer: CCN4 (WISP1) has tumor suppressive effects and expression is inversely correlated with poor prognosis, whilst CCN5 is linked with aggressive disease[10] and estrogen receptor positivity.[11] CCN6 has been associated with neoplastic progression[12] and conversely, as

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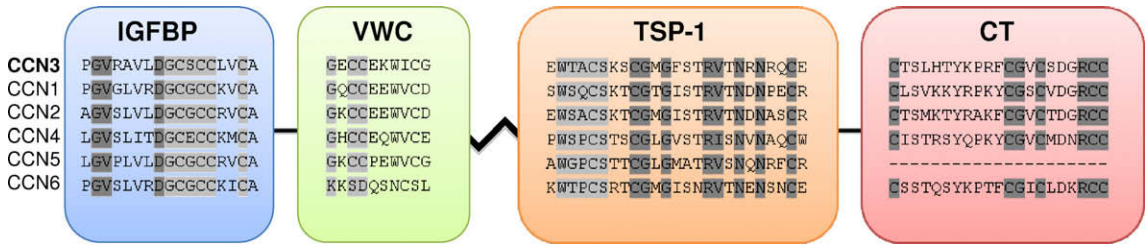

Figure 1. Structure of the CCN protein family. CCN proteins are comprised of a signal peptide followed by four structurally distinct domains; insulin-like growth factor binding protein domain (IGFBP), von Willebrand type C repeat (VWC), thrombospondin type I domain (TSP-1) and cysteine-rich carboxyl terminal (CT). CCN proteins share 60% amino acid homology and contain highly conserved residues (dark grey) and sequences associated with domain function (light grey). CCN proteins 1–4 and CCN6 retain all of the structural modules whereas the CT module is absent in CCN5. (Partial domain sequences are shown to illustrate sequence homology).

a tumor suppressor in inflammatory breast cancer.[13] CCN4 promotes chondrocyte proliferation and osteoblastic differentiation in bone marrow[14] and the loss of CCN5 is connected with cellular transformation in pancreatic cancer.[15]

CCN3 protein structure

Full-length CCN3 protein (48–50 kDa) comprises a signal peptide followed by the 4 structural domains. The IGFBP, VWC and TSP-1 domains are associated with protein binding and the CT domain promotes oligomerization. CCN3 is characteristically expressed in the cytoplasm and accumulates in the external environment owing to the signal peptide (SP).

Several CCN3 isoforms have been identified and since only one *CCN3* transcript has been observed this suggests that these proteins arise by proteolytic cleavage. The 'hinge' region between the VWC and TSP-1 domains, appears susceptible to proteolytic cleavage and generates protein with nuclear accessibility. Characteristic 35 kDa and 28 kDa isoforms have been well documented and are reported to be truncated protein forms lacking N-terminal modules.[16,17] Yeast 2 hybrid analysis has shown CCN3 interaction with the Rpb7 subunit of RNA polymerase II, providing further evidence for the potential of CCN3 to act as a transcriptional regulator. Soon after, CCN3 was also shown to bind Notch1,[18] Integrins,[19] Fibulin 1c,[20] BMP-2[21] and co-localise with Connexin 43 at gap junctions.[22]

There are further layers of complexity. CCN3 may be expressed as a number of isoforms with distinctly different properties; full-length CCN3 causes growth inhibition of chicken embryonic fibroblasts whereas an amino-truncated form induces morphological transformation.[16,17] Nuclear accumulation and transcriptional regulation is proposed to be a key element in the balance between CCN3 pro- and anti-proliferative properties.[23] The majority of studies in malignant tissues have been conducted using a CCN3 antibody that was raised to a peptide within the CT domain (K19M) or using primers designed to pick up *CCN3* gene expression. Neither method will distinguish between expression of full-length and truncated isoforms. Since the recent generation of domain-specific antibodies, a distinct truncated form lacking the TSP-1 module has been identified in Wilm's Tumors.[24] It is likely that CCN3 isoforms will play a predominant role in oncogenesis and malignancy.

CCN3 and Hematology

CCN3 expression has been detected at various stages of commitment within the myeloid lineage; CD34+ progenitors and mononuclear cells (MNC) derived from normal bone marrow (NBM) have been shown to express CCN3.[25] Recent microarray studies support these findings and show *CCN3* expression within early myeloid and more committed progenitors (NCBI Geoprofiles; http://www.ncbi.nlm.nih.gov/sites/entrez?db=geo&term=gds1230+AND+214321_at). *CCN3* levels increase with myeloid cell commitment and loss of 'stemness'. In contrast, Notch activity is high in hematopoietic progenitors and decreases upon differentiation.[26] Modulation of both Notch and CCN3 may be key in balancing self-renewal and differentiation within hematopoiesis. A study designed to identify conserved genes in hematopoietic stem cells showed that *CCN3* was highly expressed in the most primitive adult progenitors, yet expression was low in progenitors derived from cord blood (CB).[27] These studies highlight distinct differences in hematopoietic stem cells derived from different origins which are clearly programmed and regulated in a discrete manner.

CCN3 in Chronic Myeloid Leukemia

CCN3 expression is reduced as a consequence of BCR–ABL kinase activity in CML[25] (Fig. 2A and B) and (NCBI Geoprofiles; http://www.ncbi.nlm.nih.gov/sites/entrez?db=geo&term=gds2342+AND+214321_at). In CML cell lines, *CCN3* expression is low or undetectable and increases in response to treatment with Imatinib or short interfering RNA designed to abrogate BCR–ABL expression.[25] Forced CCN3 expression or treatment with recombinant CCN3 in CML cells was growth inhibitory and restored the induction of apoptosis. In primary CML CD34+ cells, BCR–ABL not only reduced CCN3 expression but also increased its secretion from the cell. BCR–ABL regulation of *CCN3* transcription and export from the cell provides a mechanism that is advantageous to the malignant clone to evade growth regulation. Impaired expression of CCN3 is likely to give rise to abnormalities within the bone marrow accruing to the CML phenotype. Changes in CCN3 expression appear to be dependent on BCR–ABL activity and not simply a reflection of myeloproliferation,[25] hence association of CCN3 with other leukemias has not been reported.

CCN3 as an agent for naïve stem cell expansion

In contrast to progenitors from adult bone marrow, primitive progenitors from human cord blood (CB) are stimulated by CCN3.[28] The disparity may be due to the naïve nature of these cells in contrast to adult progenitors, or perhaps distinct molecular programming that is associated with cell origin. CCN3 is required to maintain long-term culture initiating cell cultures (LTC-IC) from naïve progenitors and the self-renewal capacity was linked with Notch activation. CCN3 was required for the development of stem, myelomonocytic and erythroid lineages and contributed to the functional development of B cells. CCN3 conferred selective engraftment potential in NOD SCID mice and similar to the case for melanoma, was linked with *p21*, the downstream target of *p53*.[28]

CCN3 regulation of bone marrow cells

In addition to hematopoietic stem cells (HSC), the bone marrow houses two other types of stem cell; Mesenchymal stem cells