Impacts of a new transcription factor family: mammalian GCM proteins in health and disease

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GCM proteins constitute a small transcription factor family with a DNA-binding domain exhibiting a novel fold composed of two subdomains rigidly held together by coordination of one of two structural zinc cations. In all known cases, GCM proteins exert the role of master regulators: the prototypical family member determines gliogenesis in Drosophila melanogaster, whereas mammalian GCM proteins orchestrate divergent aspects of development and physiology in placenta, kidney, thymus, and parathyroid gland. Recent data point to an involvement of GCM proteins in different pathological contexts, such as preeclampsia, hyper- or hypoparathyroidism, and parathyroid gland tumors.

The story of glial cells missing (gcm) started with a screen for axonal pathfinding mutants in Drosophila melanogaster (Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). The identified prototypic GCM (also called Glide; glial cell deficient) turned out to be the earliest binary switch known to be necessary for glial cell development in the fruit fly. Surplus of GCM/Glide instructs neural precursor cells to differentiate into glial cells, whereas in the absence of GCM/Glide neutral precursors predominantly mature to neurons. For details of GCM function in Drosophila, we refer the reader to several recent reviews (Jones, 2001; Van De Bor and Giangrande, 2002). GCM proteins encode transcription factors with a new type of zinc-containing DNA-binding domain, a so-called GCM domain, that recognizes the DNA sequence 5'-ATGCCGGTG-3' or variations thereof (Akiyama et al., 1996; Schreiber et al., 1997, 1998; Cohen et al., 2002). The GCM domain represents the structural hallmark and the only region of significant homology of the different GCM family members. It is assembled from a large and a small subdomain, both rich in β-pleated sheets. One of the two zinc cations tethers the two subdomains together, while the other is located at the periphery of the small subdomain. A five-stranded β-sheet from the large domain protrudes into the major groove perpendicularly to the DNA axis and thereby defines a novel use of β-sheets in DNA recognition (Cohen et al., 2003).

All GCM proteins known to date are listed in Fig. 1. In Drosophila, a second gcm gene was identified (Kammerer and Giangrande, 2001; Alfonso and Jones, 2002). This gcm2/glide2 gene is the result of a recent gene duplication event and assists gcm/glide in its function during gliogenesis. In the sea urchin Strongylocentrotus purpuratus, SpGcm was identified as a Notch-dependent factor (Calestani et al., 2003). Interestingly, Notch signaling also accounts for gcm/glide activation in Drosophila’s nervous system, and thus might be a common denominator in gcm regulation. A GCM protein has also been identified and characterized in Gallus gallus (Hashemolhosseini et al., 2004). Current sequencing efforts have revealed additional GCM proteins in the genomes of Xenopus laevis, Fugu rubripes, and Danio rerio (unpublished data).

GCM in mammals
In search for regulators of mammalian gliogenesis, two GCM proteins, named GCMa/Gcm1 and GCMb/Gcm2, were identified (Akiyama et al., 1996; Kim et al., 1998; Schreiber et al., 1998). No other functional gcm genes have been detected in the human and mouse genome. Initially, overexpression studies supported a function of mammalian GCM proteins in nervous system development. Thus, GCMa/Gcm1 disrupted neurogenesis or induced gliogenesis when ectopically expressed in the nervous system of Drosophila and mammals (Kim et al., 1998; Reifegerste et al., 1999; Nait-Oumesmar et al., 2002; Iwasaki et al., 2003). However, all studies reported to date failed to detect significant amounts of GCMa/Gcm1 or GCMb/Gcm2 in the mammalian nervous system. If at all detected in embryonic brain, expression rates were found orders of magnitude lower than in other tissues. In GCMa/Gcm1 knockout mice that express a β-galactosidase marker instead of GCMa/Gcm1, no β-galactosidase–positive cells were detected in the brain (Hashemolhosseini et al., 2002).

Expression of mammalian GCM proteins was highest in tissues other than the nervous system. During mouse development from embryonic day (E) 7.5 to E17.5 GCMa/
Gcm1 is expressed in the fetal part of the placenta and defines a unique subset of trophoblast cells (Basyuk et al., 1999). Knockout mouse models demonstrate embryonic lethality in the absence of GCMa/Gcm1 around E10 (Anson-Cartwright et al., 2000; Schreiber et al., 2000). GCMa/Gcm1 plays a pivotal role in placental labyrinth development where it is required for placental branching morphogenesis. After GCMa/Gcm1 deletion, the allantois is still able to grow toward and contact the chorion, but subsequent steps of placental labyrinth formation are affected so that the failure to supply the embryo with sufficient amounts of nutrients and oxygen leads to embryonic death. Interestingly, the GCM protein identified from chicken is also found in the egg’s chorion (Hashemolhosseini et al., 2004). Thus, its localization is reminiscent of the spatial expression profile of GCMa/Gcm1. Apart from the fetal part, GCMa/Gcm1 expression is selectively turned on at late embryonic stages in the thymus and perinatally in the kidney (Hashemolhosseini et al., 2002). In both of these organs, GCMa/Gcm1 is expressed throughout adulthood. In the thymic cortex a few clusters of ~30 GCMa/Gcm1-positive cells appear first at E16.5. During development and adult stages, the number of clusters increases with a concomitant decrease of the number of cells to two to three cells per cluster. In the kidney of mice, GCMa/Gcm1 expression was detected specifically in cells of the S3 segment of proximal tubules. Because renal GCMa/Gcm1 expression occurs after kidney development is almost completed, GCMa/Gcm1 is likely involved in kidney physiology.

Although not much is known about mechanistic aspects of GCMa/Gcm1 function in the kidney, several aspects of its regulation and function have been studied in the placenta. Integrin-α4 was identified by antibody-blocking experiments as an upstream component of the GCMa-signaling cascade in placental trophoblasts (Stecca et al., 2002). Once induced, GCM proteins must be able to efficiently translocate to the nucleus to perform their function as transcription factors. For that matter, all GCM proteins possess nuclear localization signals. For some GCM proteins like GCMb/Gcm2 or the prototypical GCM/Glide, these conform to the classical bipartite nuclear localization motif. In others like GCMa/Gcm1 or chick GCM, nuclear localization is mediated by regions without resemblance to known nuclear localization signals (Hashemolhosseini et al., 2003). Nuclear import was counteracted in GCMa/Gcm1 by an amino terminal nuclear export activity. In fact, several GCM proteins including GCMa/Gcm1 have also been found in the cytosol (Bernardoni et al., 1999; Nait-Oumesmar et al., 2000). Thus, nucleocytoplasmic shuttling might be a conserved feature within this family of transcription factors and might offer control of their activity either through sequestration or cytoplasmic posttranslational modification.

The transactivation capacity of all mammalian GCM proteins can be attributed to two transactivation domains compared with one in the prototypical Drosophila GCM/Glide protein (Schreiber et al., 1998). Furthermore, GCM proteins contain PEST motifs within their primary structure that convey short half-lifes and low protein stability (Tuerk et al., 2000) and influence target gene activation. For GCMa/Gcm1, two placental target genes are known (Yamada et al., 1999; Yu et al., 2002). These genes are human aromatase and human syncytin. In the case of the aromatase gene, GCMa/Gcm1 binds to a minimal enhancer, which is essential for placenta-specific transcription. In the case of syncytin, GCMa/Gcm1 induces the promoter activity of the long-terminal repeats of a syncytin-harboring retrovirus integrated into the human genome and specifically drives endogenous syncytin expression in human trophoblast lines. Syncytin in humans can mediate fusion of cytotrophoblasts into the syncytiotrophoblast layer, and syncytin expression is tightly regulated to maintain a functional syncytiotrophoblast layer. However, mice do not possess a retroviral encoded syncytin, so that GCMa/Gcm1-dependent syncytin expression cannot explain syncytiotrophoblast formation in mice. This finding suggests additional placental GCMa/Gcm1-inducible factors for labyrinth formation in the mouse.

**Figure 1. Overview and domain topology of GCM proteins.** Numbers to the right indicate amino acid residues in each GCM protein. To avoid superimposing multiple domains in the same GCM protein, one of the two is shown at half height. At the right, the expression site of each GCM protein is listed. Note the presence of two transactivation domains for mammalian and chick GCM proteins. For GCM2/Glide2, xeGCM, and SpGcm, the GCM domain is the only characterized domain. At the bottom, differently patterned squares corresponding to different functional domains are shown (DBD, DNA-binding domain; TA, transactivation domain; NLS, nuclear localization signal; NES, nuclear export signal; PEST, proline-glutamine-serine-threonine rich motif; ID, inhibitory domain).
GCMa/Gcm1 is likely to activate its target genes in combination with interacting transcription factors. Recently, Pitx2 has been identified as the first protein interacting with GCMa/Gcm1 in placenta and kidney (unpublished data).

The expression of the second mammalian GCM gene, GCMb/Gcm2, is restricted to parathyroid glands (Kim et al., 1998). 30% of GCMb/Gcm2-deficient mice die shortly after birth owing to severe hypocalcaemia. The remaining were viable, fertile, and had milder hypocalcaemia associated with birth owing to severe hypocalcaemia. The remaining were viable, fertile, and had milder hypocalcaemia associated with hyperphosphataemia and increased calcium elimination in the urine without evidence of renal participation, altogether features characteristic for hypoparathyroidism (Günther et al., 2000). In agreement, GCMb/Gcm2-deficient mice lack parathyroid glands but show normal parathyroid hormone serum levels. In the same study, GCMa/Gcm1-mediated parathyroid hormone production in the thymus was uncovered as an auxiliary regulatory mechanism for calcium homeostasis in the absence of parathyroid glands.

In humans and mice, parathyroid glands as well as the thymus arise from the pharyngeal pouches. GCMb/Gcm2 and Foxn1 are expressed in a complementary fashion in the common primordium, thereby dividing it into separate domains that prefigure parathyroid and thymus (Gordon et al., 2001). Transcription factors that determine cell fate in the common primordium and subsequent development of thymus and parathyroid glands have recently been identified. GCMb/Gcm2 expression is reduced in Pax1<sup>−/−</sup> mutants by E11.5, and further reduced or absent in Hoxa3<sup>+/−</sup>/Pax1<sup>−/−</sup> double mutants, indicating that GCMb/Gcm2 is under control of these transcription factors (Su et al., 2001).

GCM in disease

GCM proteins so far turned out to exert crucial roles as master regulators in different developmental contexts. Therefore, it would be surprising if they had no significance in pathological circumstances. Concerning GCMa/Gcm1’s action in the placenta, GCMa/Gcm1 is the first transcription factor capable of initiating syncytiotrophoblast formation. Accordingly, GCMa/Gcm1 might play a role in placental diseases such as preeclampsia and intrauterine growth restriction. These conditions complicate 5–10% of human pregnancies and result in fetal to neonatal mortality and morbidity (Anson-Cartwright et al., 2000). Indeed, decreased placental GCMa/Gcm1 expression has been observed in preeclampsia (Chen et al., 2004). GCMa/Gcm1 expression was reduced in a statistically significant number of preeclamptic placentae compared with gestational age-matched controls. In this work, changes in the expression of syncytiotrophoblast were not analyzed. One would assume that transcript levels of this GCMa/Gcm1 target gene are also diminished.

Because GCMb/Gcm2 was identified as a master regulatory gene of parathyroid gland development, alterations of its levels or activity might contribute to pathological states of this organ. Homozygocity for a large intragenic deletion within the GCMb/Gcm2 gene was identified as the cause for autosomal recessive hypoparathyroidism in a 5-wk-old patient suffering from generalized seizures (Ding et al., 2001). Recently, another case of familial hypoparathyroidism has been observed. In this case, a single amino acid change in the GCM domain of GCMb/Gcm2 that results in a transcriptionally significantly less active GCMb/Gcm2 was identified as the underlying cause of the disease (unpublished data). Thus, loss of GCMb/Gcm2 function might be a more common cause of hypoparathyroidism. Unexpectedly and contrary to mice, embryonic and adult thymus of healthy humans were assayed negative for both parathyroid hormone and GCMa/Gcm1 (Maret et al., 2004).

Recent data also implicate GCMb/Gcm2 in tumorogenesis. These tumors are either located in the thymus or in the parathyroid gland. Intrathymic adenomas have been reported to secrete parathyroid hormone and produce GCMb/Gcm2, suggesting that these tumors originate from misplaced parathyroid cells (Maret et al., 2004). GCMb/Gcm2 might be a diagnostic marker for these intrathymic adenomas. The amount of GCMb/Gcm2 in cells of these intrathymic adenomas was not investigated. Human GCMb/Gcm2 was also detected in several sporadic adenomas of the parathyroid gland (Kanemura et al., 1999). In these parathyroid hormone-producing adenomas, GCMb/Gcm2 was present, but expressed at significantly lower levels than in surrounding normal parathyroid tissue arguing that there is no strict correlation between GCMb/Gcm2 and parathyroid hormone expression (Correa et al., 2002). It is tempting to speculate that constant high levels of GCMb/Gcm2 expression are incompatible with proliferation and de-differentiation in an adenoma.

Concluding remarks

Both mammalian GCM proteins play crucial roles at different stages of development. GCMa/Gcm1 may have additional functions in renal physiology. It is a prime candidate for a transcription factor that regulates expression of different renal transporter genes. Due to the early embryonic lethality of a constitutive GCMa/Gcm1 knockout mouse, this has to be analyzed by generation and characterization of a conditional knockout mouse. Apart from their normal function, mammalian GCM proteins have recently been implicated in different human diseases such as preeclampsia, hyper- or hypoparathyroidism, and tumorogenesis. Further knowledge of the molecular action of mammalian GCM proteins through identification of more complete sets of target genes and binding partners might clarify mechanistic details of their contribution to disease.
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