Glycobiology vol. 11 no. 3 pp. 19R–23R, 2001

MINI REVIEW

Glypicans in growth control and cancer

Jorge Filmus

Division of Cancer Biology Research, Sunnybrook & Women's College Health Sciences Centre, and Department of Medical Biophysics, University of Toronto, 2075 Bayview Avenue, Toronto, Ontario, M4N 3M5, Canada

Accepted on December 15, 2000

The name *glypican* has been assigned to a family of heparan sulfate (HS) proteoglycans that are linked to the cell membrane by a glycosyl-phosphatidylinositol anchor. To date, six family members of this family have been identified in mammals (GPC1 to GPC6) and two in *Drosophila*. Glypicans are expressed predominantly during development, and they are thought to play a role in morphogenesis. As HS-carrying molecules, glypicans were initially considered potential regulators of heparin-binding growth factors. This has been recently confirmed by genetic interaction experiments showing that glypicans regulate *wingless* signaling in *Drosophila*. The involvement of glypicans in the *in vivo* regulation of other heparin-binding growth factors, such as fibroblast growth factors, remains to be determined. Interestingly and unexpectedly, a role for GPC3 in the regulation of insulin-like growth factors has been proposed. This hypothesis is based on the phenotype of patients with Simpson-Golabi-Behmel syndrome (SGBS), an overgrowth and dysmorphic syndrome in which the *GPC3* gene is mutated. Thus, it is possible that glypicans regulate different kinds of growth factors in a tissue-specific manner. In addition to its involvement in SGBS, down-regulation of GPC3 has been recently associated with the progression of several types of malignant tumors, including mesotheliomas and ovarian cancer. A role for GPC1 in pancreatic cancer progression has also been proposed.

Key words: heparan sulfate proteoglycans/overgrowth syndromes/glypicans.

The glypican family

Glypicans are a family of heparan sulfate proteoglycans (HSPGs) that are linked to the exocytoplasmic surface of the plasma membrane by a glycosyl-phosphatidylinositol (GPI) anchor. The size of the core proteins of glypicans is similar (60 to 70 kDa), and, as expected, they all contain an N-terminal secretory signal peptide and a hydrophobic domain in the C-terminal region required for the insertion of the GPI anchor. Although the degree of amino acid homology between most glypicans is moderate (Veugelers *et al.*, 1999), the location of 14 cysteine residues is conserved, suggesting the existence of a highly similar three-dimensional structure. Another shared feature of glypicans is the location of the heparan sulfate (HS) insertion sites, which seems to be restricted to the last 50 amino acids in the C-terminus, placing the HS chains close to the cell membrane (Saunders *et al.*, 1997).

To date, six members of the glypican family (GPC1 to GPC6) have been identified in mammals (Table 1), and two in *Drosophila* (Nakato *et al.*, 1995; Baeg *et al.*, 2001). The greater degree of homology between the primary amino acid sequences of GPC3 and GPC5 suggests that these glypicans share unique structural features that distinguish them from the other family members (Paine-Saunders *et al.*, 1999). In addition, it is interesting to note that GPC3 and GPC4 are clustered on chromosome Xq26 (Veugelers *et al.*, 1998), and GPC5 and GPC6 on chromosome 13q32 (Veugelers *et al.*, 1999). This suggests that the glypican family arose as a result of gene duplication.

In general, glypicans are expressed predominantly during development. Their expression levels change in a stage- and tissue-specific manner, suggesting that glypicans are involved in the regulation of morphogenesis (Li *et al.*, 1997; Saunders *et al.*, 1997; Litwack *et al.*, 1998).

The function of glypicans

During the last few years it has been clearly established that cell-surface HSPGs are required for the optimal activity of heparin-binding growth factors, such as fibroblast growth factors (FGFs) and Wnts (Yayon *et al.*, 1991; Schlessinger *et al.*, 1995; Reichsman *et al.*, 1996; Binari *et al.*, 1997). In the case of FGF2, a model has been proposed in which the HS chains interact with both the ligand and the high-affinity FGF receptor. This interaction increases FGF2–FGF receptor binding and also promotes FGF receptor dimerization (Venkataraman *et al.*, 1999; Schlessinger, 2000; Stauber *et al.*, 2000).

In addition to glypicans, there are other types of HSPGs present on the cell surface. Unfortunately, the experiments that established the requirement of HSPGs for optimal FGF signaling *in vivo* did not identify the specific HSPGs involved (Lin *et al.*, 1999b). Thus, although it is very clear that in tissue cultured cells glypicans can bind FGF2 and increase its mitogenic activity (Steinfeld *et al.*, 1996; Song *et al.*, 1997; Bonneh-Barkay *et al.*, 1997), there is still no proof that glypicans are required for FGF activity *in vivo*. With regard to Wnt signaling, on the other hand, an *in vivo* role for glypicans has been clearly demonstrated. The experimental evidence was generated by studying the role of *dally*, a *Drosophila* glypican (Lin and Perrimon, 1999; Tsuda *et al.*, 1999). Reduction of *dally* expression as a result of mutation or the use of RNA interference is associated with segment polarity defects similar to the ones

Table 1. Glypican family

Name	Original Designation	Expression in Embryo	Expression in Adult	Reference
Glypican 1	Glypican	Bone, bone marrow, muscle epidermis, kidney	Most tissues	David *et al.*, 1990; Litwack *et al.*, 1994
Glypican 2	Cerebroglycan	Nervous system	Not detected	Stipp *et al.*, 1994; Ivins *et al.*, 1997
Glypican 3	OCI-5	Most tissues	Ovary, mammary gland, mesothelium, lung, kidney	Filmus *et al*., 1998; Pellegrini *et al.*, 1998; Li *et al*., 1997; Filmus (unpublished observations)
Glypican 4	K-glypican	Brain, kidney, lung	Most tissues	Watanabe *et al.*, 1995; Veugelers *et al.*, 1998; Siebertz *et al.*, 1999
Glypican 5		Brain, lung, liver, kidney, limb	Brain	Veugelers *et al.*, 1997; Saunders *et al.*, 1997
Glypican 6		Many tissues, including liver and kidney	Many tissues including ovary, kidney, liver, and intestine	Paine-Saunders *et al.*, 1999; Veugelers *et al.*, 1999

caused by the loss of *wingless* activity (Lin and Perrimon, 1999; Tsuda *et al.*, 1999). Further genetic interaction experiments suggest that *dally* regulates *wingless* extracellular interactions (Baeg and Perrimon, 2000), which is consistent with a previous report indicating that cell-surface HS is required for optimal Wnt signaling (Reichsman *et al.*, 1996). The existence of a *dally-like* (*dly*) gene in *Drosophila* has been recently reported (Baeg *et al.*, 2001). Although *dly* mutants are not available yet, results of RNA interference experiments suggest that *dly* can also regulate *wingless* activity.

Genetic interaction experiments have also implicated glypicans in the regulation of the activity of bone morphogenetic proteins (BMPs). Work in *Drosophila* with *dally* mutants has shown that this glypican regulates the signaling of *dpp* (a *Drosophila* BMP) in specific tissues during development (Jackson *et al.*, 1997; Tsuda *et al.*, 1999). Furthermore, when GPC3-deficient mice are mated with BMP4 heterozygote mice, the offspring displays polydactyly and rib malformations with high penetrance (Paine-Saunders *et al.*, 2000). These abnormalities are not observed in either parental strain.

Taken together, the studies described above provide strong evidence in favor of genetic interactions between Wnt and *dpp*/BMP pathways, and glypicans. However, the biochemical basis for these interactions remains to be defined.

The peculiar case of GPC3

In 1996 Pilia *et al*. reported that *GPC3* is mutated in patients with the Simpson-Golabi-Behmel syndrome (SGBS) (Pilia *et al.*, 1996). This is an X-linked disorder characterized by pre- and postnatal overgrowth and a broad spectrum of clinical manifestations that vary from a very mild phenotype in carrier females to infantile lethal forms in some males (reviewed in Neri *et al.*, 1998). The list of clinical manifestations of SGBS can include a distinct facial appearance, macroglossia (enlarged tongue), cleft palate, syndactyly (incomplete separation of digits), polydactyly (more digits than normal), supernumerary nipples, cystic and dysplastic kidneys, congenital heart defects, rib and vertebral abnormalities, and umbilical/inguinal hernias.

Most of the *GPC3* mutations identified to date are point mutations or microdeletions encompassing a varying number of exons (Hughes-Benzie *et al.*, 1996; Lindsay *et al.*, 1997; Veugelers *et al.*, 2000). Given the lack of correlation between phenotype and the location of the mutations, it has been proposed that SGBS is caused by a nonfunctional GPC3 protein, with additional genetic factors responsible for the intra- and interfamilial phenotypic variation (Hughes-Benzie *et al.*, 1996). Strong support for this hypothesis has been provided by the generation of GPC3-deficient mice (Cano-Gauci *et al.*, 1999; Paine-Saunders *et al.*, 2000). These mice display several of the abnormalities found in SGBS patients, including developmental overgrowth (Figure 1) and cystic and dysplastic kidneys. Starting from early stages of development there is a persistent increase in the proliferation rate of epithelial cells in the ureteric bud/collecting system (Cano-Gauci *et al.*, 1999). This observation supports the idea that GPC3 is a negative regulator of cell proliferation, which is obviously consistent with the general overgrowth observed in the SGBS patients and the GPC3-deficient mice.

Some of the clinical features of the SGBS patients, such as syndactyly and the presence of multiple nipples, suggest that

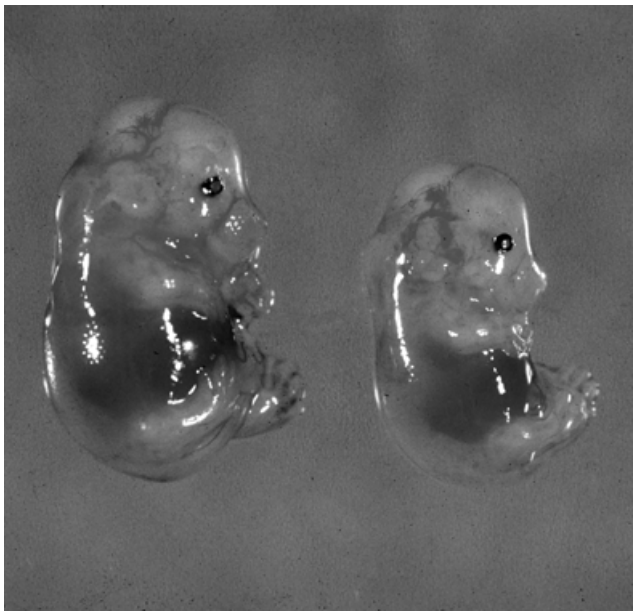

Fig. 1. Developmental overgrowth in GPC3-deficient mice. Representative picture of day 14 embryos. Left: GPC3-deficient; Right: Wild type.

GPC3 may act to regulate cell survival in certain tissues during development. Our laboratory recently provided experimental support to this hypothesis by demonstrating that GPC3 can induce apoptosis in a cell type–specific manner (Duenas Gonzales *et al.*, 1998).

SGBS shares some clinical features with the Beckwith-Wiedemann syndrome (BWS), another overgrowth syndrome (Weng *et al.*, 1995). Because overexpression of insulin-like growth factor II (IGF-II) is thought to be one of the contributing factors to BWS (Weksberg and Squire, 1997), it has been proposed that GPC3 negatively regulates IGF-II activity by competing for IGF-II binding with the signaling receptor and that the loss-of-function mutations of GPC3 are equivalent to overexpression of IGF-II (Weksberg *et al.*, 1996). Further support for this idea was provided by the generation of mice overexpressing IGF-II (Eggenschwiler *et al.*, 1997). In addition to the phenotypic features of BWS, these mice display skeletal defects that are typical of SGBS. It has to be noted, however, that the SGBS patients and the GPC3-null mice display severe kidney abnormalities that are not found in the IGF-II transgenic mice.

Another piece of evidence suggesting an involvement of GPC3 in IGF-II signaling comes from the finding that IGF-II receptor (IGF2A)–deficient mice display the same degree of developmental overgrowth than the GPC3-null mice (Wang *et al.*, 1994; Lau *et al.*, 1994). The IGF2R is a well-characterized negative regulator of IGF-II. It binds this growth factor and down-regulates its activity by endocytosis and degradation (Ludwig *et al.*, 1996). Thus, the IGF2R-deficient mice display increased levels of IGF-II in blood and tissues. In the case of the GPC3-null embryos, however, no significant alteration in IGF-II levels has been found (Cano-Gauci *et al.*, 1999). Furthermore, no direct interaction between IGF-II and GPC3 has been detected (Song *et al.*, 1997). It can be concluded, therefore, that if GPC3 inhibits IGF-II signaling, it does so by a mechanism that is fundamentally different than that used by the IGF2R.

Given the complex clinical features of SGBS and the differences in the kidney phenotype between the IGF-II transgenic and the GPC3-null mice, it is also possible that GPC3 regulates other growth factors in addition to IGF-II. As discussed above, there is already one report demonstrating a genetic interaction between BMP4 and GPC3 (Paine-Saunders *et al.*, 2000). In addition, it is important to note that there is some experimental evidence suggesting that the HS chains are not required for all the activities of GPC3 (Duenas Gonzales *et al.*, 1998). Thus, it can be proposed that the protein core of GPC3 interacts with other molecule(s) independent of the HS chains. In addition, because GPC3, like other glypicans, can be secreted (Filmus *et al.*, 1995), it is possible that the secreted form of GPC3 may act via a mechanism distinct from that of the cell surface form.

Glypicans and cancer

Because GPC3 is an inhibitor of cell proliferation and can induce apoptosis in certain types of tumor cells (Duenas Gonzales *et al.*, 1998; Cano-Gauci *et al.*, 1999), recent reports indicating that GPC3 expression is down-regulated in tumors from different origin were not surprising. Lin *et al*. showed that, although GPC3 is expressed in normal ovary, its expression is undetectable in a significant proportion of ovarian cancer cell lines (Lin *et al.*, 1999a). In all cases where GPC3 expression was lost, the *GPC3* promoter was hypermethylated, and no mutations were found in the coding region. GPC3 expression was restored by treatment with a demethylating agent. In addition, the authors demonstrated that ectopic expression of GPC3 inhibits colony-forming activity in several ovarian cancer cell lines.

Another report associating GPC3 with cancer was originated from a differential mRNA display study on normal rat mesothelial cells and mesothelioma cell lines (Murthy *et al.*, 2000). In this study it was found that GPC3 was consistently down-regulated in the tumor cell lines. Moreover, a similar down-regulation was found in primary rat mesotheliomas and in cell lines derived from human mesotheliomas. Similarly to ovarian cancer, no mutations in the GPC3 coding sequence were found, but most of the cell lines displayed aberrant methylation in the GPC3 promoter region. As reported previously (Duenas Gonzales *et al.*, 1998), this study showed that ectopic expression of GPC3 in mesothelioma cell lines inhibits their colony-forming activity.

Interestingly, another differential mRNA study comparing normal liver and human hepatocellular carcinomas found that, although GPC3 is not expressed in the liver, the expression of this glypican is up-regulated in the majority of tumors (Hsu *et al.*, 1997). In a similar manner, in our laboratory we found that, though normal colon does not express GPC3, a significant proportion of colorectal tumors do (unpublished observations). Because GPC3 is highly expressed in embryonic liver and intestine and is silenced in the corresponding normal adult tissues, these results suggest that in these organs GPC3 is behaving as an oncofetal protein. In general, oncofetal proteins do not seem to play a critical role in tumor progression, but they have been used as tumor markers or as targets for immunotherapy (Coggin, 1992; Matsuura and Hakomori, 1985). In this regard, Hsu *et al.* (1997) reported that GPC3 is more frequently upregulated in hepatocarcinomas than α-fetoprotein, another oncofetal protein that has been extensively used in the clinic as a tumor marker (Beasley *et al.*, 1981) and that is thought to be a potential target for immunotherapy (Vollmer *et al.*, 1999). It remains to be seen whether the oncofetal behavior of GPC3 can be exploited for clinical use.

In summary, depending on the tissue, GPC3 displays a very different pattern of expression during tumor progression. In cancers originated from tissues that are GPC3-positive in adults, the expression of GPC3 is reduced during tumor progression; this reduction seems to play a role in the generation of the malignant phenotype. On the other hand, in tumors originated from tissues that only express GPC3 in the embryo, GPC3 expression tends to reappear on malignant transformation. Whether GPC3 reexpression plays a role in the progression of these tumors is still not known. We speculate that this tissue-specific differences are due to the fact that GPC3 is regulating different growth and survival factors in each tissue.

Another connection between glypicans and tumor progression has been established by a recent study that showed that GPC1 expression is significantly increased in a large proportion of pancreatic tumors (Kleef *et al.*, 1998). Furthermore, this study also reported that transfection of antisense GPC-1 inhibited the mitogenic response of cultured pancreatic cancer cells to FGF2 and heparin-binding EGF-like growth factor (HB-EGF) (Kleef

et al., 1998), and decreased the tumorigenicity of the transfected cells in nude mice (Kleeff *et al.*, 1999). The molecular basis of the reduced tumorigenicity remains to be determined, although it is certainly possible that a reduced response to FGF2 or HB-HGF is the cause of this reduction.

Perspective

A significant amount of genetic evidence has been generated in the last few years demonstrating that glypicans play a critical role in the regulation of cell proliferation and survival, particularly during development and malignant transformation. This role of glypicans seems to be based on their capacity to modulate the activity of various growth and survival factors. The challenge in the future will be to define more precisely which growth and survival factors are regulated by each glypican *in vivo* and to uncover the precise biochemical basis of such regulation.

Acknowledgments

I thank Norman Rosemblum and Howard Song for critically reviewing this manuscript and Sophie Ku for assistance in its preparation. The work in my laboratory has been supported by the Medical Research Council of Canada, the National Cancer Institute of Canada, and the March of Dimes.

Abbreviations

BMP, bone morphogenetic protein; BWS, Beckwith-Wiedemann syndrome; *dly*, *dally-like*; FGF, fibroblast growth factor; GPI, glycosyl-phosphatidylinositol; HB-GBF, heparin-binding; EGF, epidermal growth factor; HS, heparan sulfate; HSPG, heparan sulfate proteoglycan; IGF-II, insulin-like growth factor II; IGF2R, IGF-II receptor; SGBS, Simpson-Golabi-Behmel syndrome.

References

Baeg, G.H., Lin, X., Khare, N., Baumgartner, S., and Perrimon, N. (2001) Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. *Development*, **126**, 87–94.

Baeg, G.H. and Perrimon, N. (2000) Functional binding of secreted molecules to heparan sulfate proteoglycans in *Drosophila*. *Curr. Opin.Cell Biol.*, **12**, 575–580.

Beasley, R.P., Hwang, L.Y., Lin, C.C., and Chien, C.S. (1981) Hepatocellular carcinoma and hepatitis B virus: a prospective study of 22,707 men in Taiwan. *Lancet*, **2**, 1129–1132.

Binari, R.C., Staveley, B.E., Johnson, W.A., Godovarti, R., Sasisekharan, R., and Manoukian, A.S. (1997) Genetic evidence that heparin-like glycosaminoglycans are involved in wingless signaling. *Development*, **124**, 2623–2632.

Bonneh-Barkay, D., Shlissel, M., Berman, B., Shaoul, E., Admon, A., Vlodavsky, I., Carey, D.J., Asundi, V.K., Reich-Slotky, R., and Ron, D. (1997) Identification of glypican as a dual modulator of the biological activity of fibroblast growth factors. *J. Biol. Chem.*, **272**, 12415–12421.

Cano-Gauci, D.F., Song, H., Yang, H., McKerlie, C., Choo, B., Shi, W., Pullano, R., Piscione, T.D., Grisaru, S., Soon, S., and others (1999) Glypican-3-deficient mice exhibit the overgrowth and renal abnormalities typical of the Simpson-Golabi-Behmel syndrome. *J. Cell Biol.*, **146**, 255–264.

Coggin, J.H. Jr. (1992) The implications of embryonic gene expression in neoplasia. *CRC Crit. Rev. Onco./Hemat.*, **5**, 37–55.

David, G., Lories, V., Decock, B., Marynen, P., Cassiman, J., and Van Den Berghe, H. (1990) Molecular cloning of a phosphatidylinositol-anchored membrane heparan sulfate proteoglycan from human lung fibroblasts. *J. Cell Biol.*, **111**, 3165–3176.

Duenas Gonzales, A., Kaya, M., Shi, W., Song, H., Testa, J.R., Penn, L.Z., and Filmus, J. (1998) OCI-5/GPC3, a glypican encoded by a gene that is mutated in the Simpson-Golabi-Behmel overgrowth syndrome, induces apoptosis in a cell line-specific manner. *J. Cell. Biol.*, **141**, 1407–1414.

Eggenschwiler, J., Ludwig, T., Fisher, P., Leighton, P.A., Tilghman, S.M., and Efstratiadis, A. (1997) Mouse mutant embryos overexpressing IGF-II exhibit phenotypic features of the Beckwith-Wiedemann and Simpson-Golabi-Behmel syndromes. *Genes & Develop.*, **11**, 3128–3142.

Filmus, J., Church, J., and Buick, R.N. (1988) Isolation of a cDNA corresponding to a developmentally regulated transcript in rat intestine. *Mol. Cell. Biol.*, **8**, 4243–4249.

Filmus, J., Shi, W., Wong, Z.M., and Wong, M.J. (1995) Identification of a new membrane-bound heparan sulfate proteoglycan. *Biochem. J.*, **311**, 561–565.

Hsu, H.C., Cheng, W., and Lai, P.L. (1997) Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. *Cancer Res.*, **57**, 5179–5184.

Hughes-Benzie, R.M., Pilia, G., Xuan, J.Y., Hunter, A.G.W., Chen, E., Golabi, M., Hurst, J.A., Kobori, J., Marymee, K., Pagon, R.A., and others (1996) Simpson-Golabi-Behmel Syndrome: genotype/phenotype analysis of 18 affected males from 7 unrelated families. *Am. J. Med. Genet.*, **66**, 227–234.

Ivins, J.K., Litwack, E.D., Kumbasar, A., Stipp, C.S., and Lander, A.D. (1997) Cerebroglycan, a developmentally regulated cell-surface heparan sulfate proteoglycan, is expressed on developing axons and growth cones. *Dev. Biol.*, **184**, 320–332.

Jackson, S.M., Nakato, H., Sugiura, M., Jannuzi, A., Oakes, R., Kaluza, V., Golden, C., and Selleck, S.B. (1997) dally, a *Drosophila* glypican, controls celular responses to the TGF-beta-related morphogen Dpp. *Development*, **124**, 4113–4120.

Kleef, J., Ishiwata, T., Kumbasar, A., Friess, H., Buchler, M.W., Lander, A.D., and Korc, M. (1998) The cell surface heparan sulfate proteoglycan glypican-1 regulates growth factor in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer. *J. Clin. Invest.*, **102**, 1662–1673.

Kleeff, J., Wildi, S., Kumbasar, A., Friess, H., Lander, A.D., and Korc, M. (1999) Stable transfection of a glypican-1 antisense construct decreases tumorigenicity in PANC-1 pancreatic carcinoma cells. *Pancreas*, **19**, 281–288.

Lau, M.M., Stewart, C.E., Liu, Z., Bhatt, H., Rotwein, P., and Stewart, C.L. (1994) Loss of the imprinted IGF2/cation-independent mannose 6-phosphate receptor results in fetal overgrowth and perinatal lethality. *Genes Dev.*, **8**, 2953–2963.

Li, M., Choo, B., Wong, Z.-M., Filmus, J., and Buick, R.N. (1997) Expression of OCI-5/Glypican 3 during intestinal morphogenesis: Regulation by cell shape in intestinal epithelial cells. *Exp. Cell Res.*, **235**, 3–12.

Lin, H., Huber, R., Schlessinger, D., and Morin, P.J. (1999a) Frequent silencing of the GPC3 gene in ovarian cancer cell lines. *Cancer Res.*, **59**, 807–810.

Lin, X. and Perrimon, N. (1999) Dally cooperates with *Drosophila* Frizzled 2 to transduce Wingless signaling. *Nature*, **400**, 281–284.

Lin, X., Buff, E.M., Perrimon, N., and Michelson, A.M. (1999b) Heparan sulfate proteoglycans are essential for FGF receptor siganlling during *Drosophila* embryonic development. *Development*, **126**, 3715–3723.

Lindsay, S., Ireland, H., O'Brien, O., Clayton-Smith, J., Hurst, J.A., Mann, J., Cole, T., Sampson, J., Slaney, S., Schlessinger, D., and others (1997) Large scale deletions in the GPC3 gene may account for a minority of cases of Simpson-Golabi-Behmel syndrome. *J. Med. Genet.*, **34**, 480–483.

Litwack, E.D., Ivins, J.K., Kumbasar, A., Paine-Saunders, S., Stipp, C.S., and Lander, A.D. (1998) Expression of the heparan sulfate proteoglycan glypican-1 in the developing rodent. *Dev. Dyn.*, **211**, 72–87.

Litwack, E.D., Stipp, C.S., Kumbasar, A., and Lander, A.D. (1994) *Neuron*al expression of glypican, a cell surface glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan, in the adult rat nervous system. *J. Neurosci.*, **14**, 3713–3724.

Ludwig, T., Eggenschwiler, J., Fisher, P., D'Ercole, A.J., Davenport, M.L., and Efstratiadis, A. (1996) Mouse mutants lacking the type 2 IGF receptor (IGF2R) are rescued from perinatal lethality in Igf2 and Igf1r null backgrounds. *Dev. Biol.*, **177**, 517–535.

Matsuura, H., and Hakomori, S-I. (1985) The oncofetal domain of fibronectin defined by monoclonal antibody FDC-6: Its presence in fibronectins from fetal and tumor tissues and its absence in those from normal adult tissues and plasma. *Proc. Natl. Acad. Sci. USA*, **82**, 6517–6521.

Murthy, S.S., Shen, T., De Rienzo, A., Lee, W.C., Ferriola, P.C., Jhanwar, S.C., Mossman, B.T., Filmus, J., and Testa, J.R. (2000) Expression of GPC3, an X-linked recessive overgrowth gene, is silenced in malignant mesothelioma. *Oncogene*, **19**, 410–416.

Nakato, H., Futch, T.A., and Selleck, S.B. (1995) The division abnormally delayed (dally) gene: a putative integral membrane proteoglycan required for cell division patterning during postembryonic development of the nervous system in *Drosophila*. *Development*, **121**, 3687–3702.

Neri, G., Gurrieri, F., Zanni, G., and Lin, A. (1998) Clinical and molecular aspects of the Simpson-Golabi-Behmel syndrome. *Am. J. Med. Genet.*, **79**, 279–283.

Paine-Saunders, S., Viviano, B.L., and Saunders, S. (1999) GPC6, a novel member of the glypican gene family, encodes a product structurally related to GPC4 and is colocalized with GPC5 on human chromosome 13. *Genomics*, **57**, 455–458.

Paine-Saunders, S., Viviano, B.L., Zupicich, J., Skarnes, W.C., and Saunders, S. (2000) Glypican-3 controls cellular responses to Bmp4 in limb patterning and skeletal development. *Dev. Biol.*, **225**, 179–187.

Pellegrini, M., Pilia, G., Pantano, S., Lucchini, F., Uda, M., Fumi, M., Cao, A., Schlessinger, D., and Forabosco, A. (1998) Gpc3 expression correlates with the phenotype of the Simpson- Golabi- Behmel syndrome. *Dev. Dyn.*, **213**, 431–439.

Pilia, G., Hughes-Benzie, R.M., MacKenzie, A., Baybayan, P., Chen, E.Y., Huber, R., Neri, G., Cao, A., Forabosco, A., and Schlessinger, D. (1996) Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. *Nature Genet.*, **12**, 241–247.

Reichsman, F., Smith, L., and Cumberledge, S. (1996) Glycosaminoglycans can modulate extracellular localization of the wingless protein and promote signal transduction. *J. Cell Biol.*, **135**, 819–827.

Saunders, S., Paine-Saunders, S., and Lander, A.D. (1997) Expression of the cell surface proteoglycan glypican-5 is developmentally regulated in kidney, limb, and brain. *Dev. Biol.*, **190**, 78–93.

Schlessinger, J. (2000) Crystal structure of a ternary FGF-FGFR-Heparin complex reveals a dual role for heparin in FGFR binding and dimerization. *Mol. Cell*, **6**, 743–750.

Schlessinger, J., Lax, I., and Lemmon, M. (1995) Regulation of growth factor activation by proteoglycans: what is the role of the low affinity receptors? *Cell*, **83**, 357–360.

Siebertz, B., Stocker, G., Drzeniek, Z., Handt, S., Just, U., and Haubeck, H.-D. (1999) Expression of glypican-4 in haematopoietic-progenitor and bone-marrow-stromal cells. *Biochem J.*, **344**, 937–943.

Song, H.H., Shi, W., and Filmus, J. (1997) OCI-5/rat glypican-3 binds to fibroblast growth factor-2 but not to insulin-like growth factor-2. *J. Biol. Chem.*, **272**, 7574–7577.

Stauber, D.J., Digabriele, A.D., and Hendrickson, W.A. (2000) Structural interactions of fibroblast growth factor receptor with its ligands. *Proc. Natl. Acad. Sci. USA*, **97**, 49–54.

Steinfeld, R., Van Den Berghe, H., and David, G. (1996) Stimulation of fibroblast growth factor receptor-1 occupancy and signaling by cell surface-associated syndecans and glypican. *J. Cell Biol.*, **133**, 405–416.

Stipp, C.S., Litwac, E.D., and Lander, A.D. (1994) Cerebroglycan: an integral membrane heparan sulfate proteoglycan that is unique to the developing nervous system and expressed specifically during neuronal differentiation. *J. Cell Biol.*, **124**, 149–160.

Tsuda, M., Kamimura, K., Nakato, H., Archer, M., Staatz, W., Fox, B., Humphrey, M., Olson, S., Futch, T., Kaluza, V., and others (1999) The cell-surface proteoglycan Dally regulates Wingless signaling in *Drosophila*. *Nature*, **400**, 276–280.

Venkataraman, G., Raman, R., Sasisekharan, V., and Sasisekharan, R. (1999) Molecular characteristics of fibroblast growth factor-fibroblast growth factor receptor-heparin-like glycosaminoglycan complex. *Proc. Natl. Acad. Sci. USA*, **96**, 3658–3663. 1–1-1999.

Veugelers, M., De Cat, B., Ceulemans, H., Bruystens, A.M., Coomans, C., Durr, J., Vermeesch, J., Marynen, P., and David, G. (1999) Glypican-6, a new member of the glypican family of cell surface proteoglycans. *J. Biol. Chem.*, **274**, 26968–26977.

Veugelers, M., De Cat, B., Muyldermans, S.Y., Reekmans, G., Delande, N., Frints, S., Legius, E., Fryns, J.P., Scrander-Stumpel, C., Weidle, B., and others (2000) Mutational analysis of the GPC3/GPC4 glypican gene cluster on Xq26 in patients with Simpson-Golabi-Behmel syndrome: identification of loss-of-function mutations in the GPC3 gene. *Hum. Mol. Genet.*, **9**, 1321–1328.

Veugelers, M., Vermeesch, J., Reekmans, G., Steinfeld, R., Marynen, P., and David, G. (1997) Characterization of glypican-5 and chromosomal localization of human GPC5, a new member of the glypican gene family. *Genomics*, **40**, 24–30.

Veugelers, M., Vermeesch, J., Watanabe, K., Yamaguchi, Y., Marynen, P., and David, G. (1998) GPC4, the gene for human K-glypican, flanks GPC3 on Xq26: deletion of the GPC3-GPC4 gene cluster in one family with Simpson-Golabi-Behmel syndrome. *Genomics*, **53**, 1–11.

Vollmer, C.M., Eilber, F.C., Butterfield, L.H., Ribas, A., Dissette, V.B., Koh, A., Montejo, L.D., Lee, M.C., Andrews, K.J., McBride, W.H., and others (1999) Alpha-fetoprotein-specific genetic immunotherapy for hepatocellular carcinoma. *Cancer Res.*, **59**, 3064–3067.

Wang, Z.Q., Fung, M.R., Barlow, D.P., and Wagner, E.F. (1994) Regulation of embryonic growth and lysosomal targeting by the imprinted Igf2/Mpr gene. *Nature*, **372**, 464–467.

Watanabe, K., Yamada, H., and Yamaguchi, Y. (1995) K-glypican: a novel GPI-linked heparan sulfate proteoglycan that is highly expressed in developing brain and kidney. *J. Cell Biol.*, **130**, 1207–1218.

Weksberg, R. and Squire, J.A. (1997) Molecular biology of Beckwith-Wiedemann syndrome. *Med. Pediatric Oncol.*, **27**, 462–469.

Weksberg, R., Squire, J.A., and Templeton, D.M. (1996) Glypicans: a growing trend. *Nature Genet.*, **12**, 225–227.

Weng, E.Y., Mortier, G.R., and Graham, J.M. (1995) Beckwith-Wiedemann syndrome. *Clin. Pediat.*, 317–326.

Yayon, A., Klagsbrun, M., Esko, J.D., Leder, P., and Ornitz, D.M. (1991) Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell*, **64**, 841–848.