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# Glypican- 3 Expression in Primary and Metastatic Neuroblastoma

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**Abstract**

Glypican-3 is an oncofetal protein found to be overexpressed in different types of tumours, such as hepatocellular carcinoma, malignant melanoma, squamous cell carcinoma of the lungs and testicular yolk sac tumour. Glypican-3 is currently emerging as a tumour marker and/or potential target for therapy of many cancers. However, there are limited studies looking for glypican-3 expression in neuroblastoma, with some evidence for loss of expression. Therefore, we sought to investigate glypican-3 expression in primary and metastatic neuroblastoma and to explore its potential as marker and/or target for development of new therapy for neuroblastoma.A total of 31 archived tissue specimens of neuroblastoma were subjected to immunohistochemical staining using a monoclonal antibody specific for glypican-3.Glypican-3 expression was compared with clinical and histological characteristics for each patient. Immunohistochemical analysis revealed for the first time overexpression of glypican-3 in 33% of neuroblastoma tumours. Its overexpression was surprisingly more predominant in metastatic tumours (71%) than in primary tumours. Glypican-3 expression was significantly correlated with disease clinical stages ($P \leq 0.05$). It was more frequently expressed in the majority of stage 4s patients and connoted poor disease prognosis. On this basis, the biological functions and molecular mechanisms underlying the overexpression of glypican-3 in neuroblastoma warrant further investigations, especially the promising use of glypican-3 for diagnostic and therapeutic purposes.

**Keywords:** Cancer, Glypican-3, Hepatocellular carcinoma, Immunohistochemistry, Neuroblastoma.

## 1. Introduction

Neuroblastoma is an embryonal, extracranial solid tumour. It arises from any primitive neural crest of sympathetic nervous system (Ishola and Chung, 2007; Park et al., 2008). It is the second most common paediatric tumour. It accounts for 10% of paediatric tumours and responsible for 15% of childhood fertilities worldwide. Because it originates from the neural crest (sympathogonia), neuroblastoma may occur anywhere in the sympathetic nervous system (Park et al., 2008). Most of primary tumours arise in the abdomen, where the adrenal gland is the most common site. Other primary locations are neck, chest and pelvis (Maris et al., 2007). Furthermore, neuroblastoma can metastasize to lymph nodes, bone marrow, bones, liver, skin and testis (Humpl, 1995). Despite the recent advances in the management of neuroblastoma, many neuroblastoma patients are dying due to uncontrolled metastasis of disease. Therefore, a new strategic approach for the treatment of neuroblastoma is needed.

Glypicans are proteins bound to external surface of plasma membrane by a glycosyl-phosphatidylinositol anchor. Six family members of glypicans have been identified, namely glypican-1 to glypican-6 in vertebrates (Filmus, 2001; Filmus and Selleck, 2001; Filmus et al., 2008; Fico et al.,

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2011). They play an important role in developmental morphogenesis and their expressions vary in a stage and tissue specific manner. It is believed that glypicans regulate cell signalling through interaction with growth factors, chemokines and structural proteins (Filmus, 2001; Iglesias et al., 2008; Fico et al., 2011).

One of the interesting members of glypicans is glypican-3. It was found to act as an onco-fetal protein in some tumours (Chan et al., 2013; Kinoshita et al., 2015). During malignant transformation, its protein and mRNA levels were abnormally re-expressed and normally silenced in some adult tissues (Filmus, 2001; Filmus and Capurro, 2008). However, the neoplastic role of glypican-3 seems to be dichotomous acting as a tumour suppressor protein in some tumours, like breast and ovary cancers, while an oncogenic protein in development of others, such as liver, colon and embryonal tumours (Lin et al., 1999; Murthy et al., 2000; Saikali and Sinnett, 2000; Zhu et al., 2001; Peters et al., 2003; Chan et al., 2013; Wang et al., 2014; Kinoshita et al., 2015). Moreover, individuals suffering from loss of function mutations for glypican-3 developed a condition called Simpson-Golabi-Behmel Syndrome (SGBS). This syndrome is characterized by malformations of pre- and postnatal overgrowth, and a wide range of abnormalities including macrocephaly, protruding jaw and tongue, widened nasal bridge, and upturned nasal tip (Cottereau et al., 2013; Pilia, Hughes-Benzie et al., 1996). Interestingly, such individuals were found at high risk for developing embryonal tumours most likely neuroblastoma and Wilm's tumour (Hughes-Benzie et al., 1992).

Recently, overexpression of glypican-3 has been reported in many types of tumours, like hepatocellular carcinoma (Zhu et al., 2001), melanoma (Nakatsura et al., 2004), lung squamous cell carcinoma (Aviel-Ronen et al., 2008), and testicular germ cell tumours (Ota et al., 2006). In hepatocellular carcinoma, glypican-3 expression was able to differentiate cases of hepatocellular carcinoma from healthy livers and benign liver lesions (Zhu et al., 2001; Nakatsura et al., 2003; Man et al., 2005; Wang et al., 2008; Zhang et al., 2012; Haruyama and Kataoka, 2016). Moreover, the serum levels of glypican-3 were used to diagnose patients with hepatocellular carcinoma but not in the case of other liver diseases or cancers (Capurro et al., 2003). Therefore, glypican-3 was suggested to be a potential serological and histological marker for the diagnosis of hepatocellular carcinoma. In neuroblastoma, there were few studies examined the glypican-3 expression in primary tumours and none of them had looked for the expression in neuroblastoma metastatic tumours. In these studies, all the neuroblastoma primary tumours demonstrated negative to weak expression of glypican-3 (Chan et al., 2013; Kinoshita et al., 2015). On this basis, the aim of the present study is to investigate glypican-3 expression in both primary and metastatic neuroblastoma tumours and to explore if glypican-3 could be valuable as a marker or a potential target for development of new therapy for neuroblastoma.

## 2. Materials and Method

### *2.1. Tissue Specimens*

Archived, formalin-fixed, paraffin-embedded tissue blocks from 31 neuroblastoma and 5 hepatocellular carcinoma patients were obtained from the surgical pathology files of King Hussein Medical Hospital, Royal Medical Services. The neuroblastoma specimens included 14 cases of primary tumours obtained from abdomen, spine, paraspine, sacrum, brain and pharynx. The rest of specimens were metastatic samples of bone marrow and lung. All personal data for specimens were kept anonymous. The present study was ethically approved by the Ethics Committee, Faculty of Medicine, Mutah University.

### *2.2. Immunohistochemistry*

Five µm-thick Formalin-Fixed and Paraffin-Embedded (FFPE) tissue sections were deparaffinised and rehydrated in graded alcohols. Sections were then subjected to heat-induced epitope retrieval in citrate buffer (10 mM citrate buffer, pH 6.0) in a microwave at 600W for 20 minutes. To block the non-specific binding of the antibodies, sections were treated with 1.5% normal goat serum for 20 minutes at room temperature. Sections were then incubated with a primary rabbit monoclonal antibody specific for glypican-3 (Abcam, UK) at concentration of (4 µg/mL) for one hour at room temperature. Following primary antibody treatment, each section was incubated with goat anti-rabbit peroxidase-conjugated secondary antibody (Vector Laboratories Ltd, Peterborough, UK) (7.5 µg/mL) for 30 minutes at room temperature. The colour reaction was developed by incubating sections with 3,3-diaminobenzidine chromogen (DAB) (Vector Laboratories Ltd, Peterborough, UK) solution for 3-5 minutes. Sections were then counterstained in Harris's haematoxylin solution mounted with coverslips using DPX medium.

The resulting slides were viewed and analyzed by using a Leica DMRB microscope (Leica DMRB, Wetzlar, Germany) with the images digitally captured and processed using a Leica MPS52 camera (Q Imaging, Germany) and the AcQuis imaging capture system (Synoptics, Cambridge, UK), respectively.

### *2.3. Analysis of Glypican-3 Expression*

Stained slides were evaluated and scored manually by two independent pathologists. The scoring system used to analyze immunohistochemical labelling of neuroblastoma clinical specimens was based on previously published studies (Gluer et al., 1998; Gluer et al., 1998). For glypican-3 expression, cells were

considered positive if they demonstrated clear membranous and/or cytoplasmic immunelabelling. Immunoreactivity for glypican-3 was scored by evaluating the number of positive tumour cells over the total number of tumour cells. The scoring results were presented as: none (0), weak (1), moderate (2) and high (3). The score 'none' indicated an absence of expression. Sections were allocated a score of 'weak' when less than 33% of cells had expression. A score of 'medium' was applied to cells which had expression on 33% to 66% of the section whilst the score 'high' represented sections which had expression on more than 66% of the cells.

### *2.4. Statistical Analysis*

The data were appropriately coded and subjected to analysis using the Nonparametric Spearman's rank order correlation coefficients using SPSS software (version 16.0, Chicago, IL). Results were considered statistically significant only if $p<0.05$.

## 3. Results

### *3.1. Demographic and Clinicopathologic Features*

All the demographic and clinical data were available for 31 children with neuroblastoma Table 1. There was a male predominance (76.7%) while female constituted only 32.3% of the patients. The most common site of primary tumours was the abdomen (61%). The majority of patients were presented with distant metastases including lymph nodes (77%), bone marrow (71%), bone (3%) and lung (3%). Twenty-two patients had metastases to both lymph nodes and bone marrow. One patient had primary tumour in the brain with no evidence of distant metastasis. Based on International Neuroblastoma Staging System (INSS), 58% of patients were clinically presented with low-stage of disease (stage 1, 2, or 4S), whereas the rest of patients were at advanced stage of disease (stage 3 or 4). According to the International Neuroblastoma Pathology Classification (INPC), twenty four patients were presented with unfavourable prognosis of disease.

**Table 1. Patient Characteristics**. INSS: International Neuroblastoma Staging System, INPC: International Neuroblastoma Pathology Classification

| Characteristics | Number/ Percentage (%) |
|---|---|
| ***Age(year)*** | |
| Mean | 5.9 |
| Gender | |
| Male | 21(76.7) |
| Female | 10 (32.3) |
| ***Site of primary tumour*** | |
| Abdomen | 19 (61) |
| Reteropharyngeal | 1 (3) |
| Spine | 3 (10) |
| Paraspine | 3(10) |
| Sacrum | 1(3) |
| Thorax | 3(10) |
| Brain | 1(3) |
| ***Sites of metastases*** | |
| Bone | 1(3) |
| Bone Marrow | 22 (71) |
| Lymph node | 24 (77) |
| Pulmonary | 1(3) |
| ***INSS stage*** | |
| 1 | 5 (17) |
| 2 | 2 (6) |
| 3 | 4 (13) |
| 4 | 9 (29) |
| 4S | 11 (35) |
| ***INPC classification*** | |
| Favorable | 7 (23) |
| Unfavorable | 24 (77) |
| ***Glypican-3 expression*** | |
| 0 | 67% |
| 1 | 33% |
| 2 | 0% |
| 3 | 0% |

### *3.2. Glypican-3 Expression in Primary and Metastatic Neuroblastoma*

Hepatocellular carcinoma tissues used as a positive control samples demonstrated a strong glypican-3 staining (Fig. 1A). Glypican-3 was only expressed in 33% of primary and metastatic neuroblastoma clinical specimens (Fig. 1). Glypican-3 staining was predominantly localised to the membrane and/or cytoplasm of tumour cells without any significant staining noted in the nucleus. All tumours expressing glypican-3 showed weak expression with less than 33% of cells expressing glypican-3. Notably, this expression was more predominant in metastatic tissues (bone marrow 71%) than in primary tumour tissues (Fig. 1G).

### *3.3. Glypican-3 Expression is Correlated with Clinical Stage*

INSS clinical staging of neuroblastoma disease was based on the extent of the primary tumour growth, local lymph node involvement, and metastases to distant sites. Glypican-3 expression was compared with INSS clinical stages of neuroblastoma. A significant correlation was found between glypican-3 expression and clinical stages ($P \leq 0.05$). Glypican-3 was more frequently expressed in patients with low stage of disease (Fig. 1B and F). The majority of tumours from patients presenting with stage 4s neuroblastoma had expression of glypican-3. Only two patients with advanced stage neuroblastoma particularly stage 4 showed glypican-3 expression (Figs. 1E and 2).

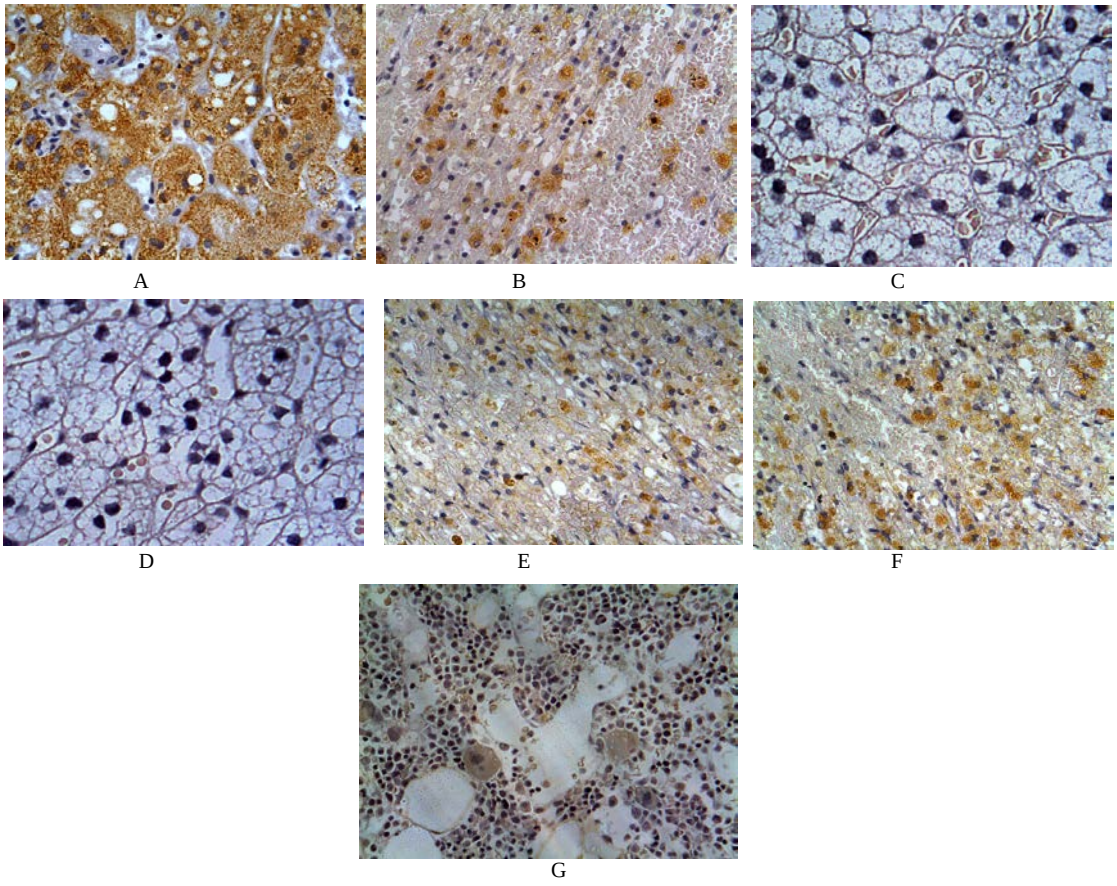


**Figure 1**. Glypican-3 expression in hepatocellular carcinoma and primary and metastatic neuroblastoma. (A)Distinct glypican-3 labelling of the cell cytoplasm seen for hepatocellular carcinoma (B, E and F) Weak glypican-3 labelling localised to the cell membrane and/ or cytoplasm was seen for neuroblastoma primary tumours in clinical stage of 1, 4 and 4s, respectively.(C and D) Negative glypican-3 expression demonstrated for neuroblastoma primary tumours in clinical stage of 3 and 4, respectively. (G)Weak glypican-3 labelling localised to the cell membrane and/ or cytoplasm was seen for neuroblastoma metastatic tumours of bone marrow a. All images are taken at the same magnification (X400).

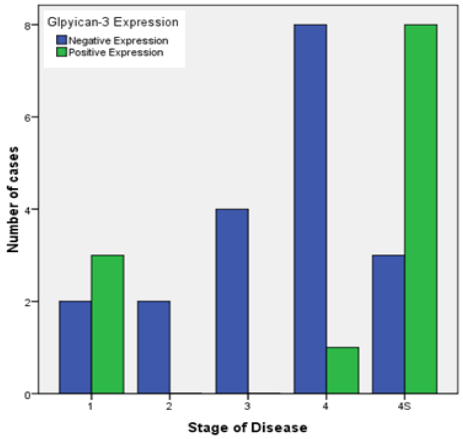


**Figure 2**. Distribution of glypican-3 expression on primary and metastatic neuroblastoma tumours and INSS clinical stage at presentation

As INPC determines the prognosis of disease in neuroblastoma patients on the basis of tumour histology and patient age, expression of glypican-3 was also compared with the INPC parameters. There was a relationship between glypican-3 expression and INPC parameters but the correlation was insignificant. Glypican-3 expression connoted unfavourable disease prognosis majorly in patients with low stage of disease (stage 4s). No statistical evidence of relationship between glypican-3 expression and age, gender and site of metastasis was detected.

## 4. Discussion

Several lines of evidence indicate the importance of glypican-3 expression in malignant tumours where it is increasingly recognized as a potential marker or a target for development of new therapy for tumours expressing glypican-3 (Capurro *et al*., 2003; Aviel-Ronen *et al*., 2008; Zynger *et al*., 2008; Tretiakova *et al*., 2015). There is growing evidence to support the potential impact of glypican-3 that contributes to malignancy in many tumours. In paediatric tumours, high levels of glypican-3 mRNA were detected in Wilm's tumour (Tretiakova *et al*., 2015), neuroblastoma cell lines (Tretiakova *et al*., 2015), hepatoblastomas (Zynger *et al*., 2008) and rhabdomyosarcomas (Thway *et al*., 2011) while low levels of glypican-3 mRNA were found in medulloblastoma and Ewing sarcoma (Saikali and Sinnett, 2000).Therefore, the oncogenic role of glypican-3 remains unclear in the light of the over-expression in certain tumours and greater risk of developing embryonic tumours secondary to glypican-3 mutations seen in the SGBS syndrome.

In the present study, for the first time we present convincing evidence of glypican-3 expression in neuroblastoma metastatic tumours and we demonstrate generally absent or weak expression in neuroblastoma primary tumours. These results came in accordant with the previous study looking for glypican-3 expression in embryonal tumours (Saikali and Sinnett, 2000). Of these, mRNA expression of glypican-3 was investigated in 10 human neuroblastoma cell lines and 4 primary tumour specimens. Glypican-3 was highly expressed in 70% of neuroblastoma cells lines and several neuroblastoma primary tumour specimens. Further studies are needed to investigate the glypican-3 mRNA and protein level in neuroblastoma metastatic tumours.

Although the glypican-3 expression was weak in neuroblastoma metastatic tumours, it correlated significantly with clinical stage. Most of the metastatic tumours expressing glypican-3 were at low stage of disease (stage 4s). As the sample size of the present study was too small, robust statistically significant correlations between glypican-3 expression and some of clinicopathologic features were not possible. However, some clear trend was apparent, particularly between expression of glypican-3 and prognosis of disease. According to INPC, all the metastatic tumours expressing glypican-3 had unfavourable disease prognosis. Further investigation is warranted using larger sample size to better delineate the relationships of glypican-3 with clinicopathologic features.

There were limited studies looking for glypican-3 expression in neuroblastoma (Chan *et al*., 2013; Kinoshita *et al*., 2015). In these studies, glypican-3 was only examined in neuroblastoma primary tumours. No studies were looking for the expression in neuroblastoma metastatic tumours. The absence of glypican-3 expression in neuroblastoma primary tumours was reported before by Chan *et al.* (2013). In that study, all the neuroblastoma primary tumours (n=136) demonstrated negative glypican-3 expression. Authors explained this observation as a result of low protein expression of glypican-3, protein conformational changes, or protein degradation (Chan *et al*., 2013). Additionally, Kinoshita and co-workers reported that glypican-3 was weakly expressed only in one patient out of 35 neuroblastoma patients (Kinoshita *et al*., 2015). This further confirms our results regarding weak or absent glypican-3 expression in neuroblastoma primary tumours.

Despite the growing evidence for the contribution of glypican-3 to tumour malignancy, the underlying mechanisms at the cellular level remain unclear. Glypican-3 seems to play an important role in controlling the functions of other molecules enhancing tumour development and metastasis. Glypican-3 was found to regulate expression of certain types of Matrix Metalloproteinases (MMPs), such as MMP-2, MMP-9 and MMP-14 in different cancers (Akutsu *et al*., 2010). Moreover, GPC3 reportedly confers oncogenicity by acting as a co-receptor for different types of growth factors, like Fibroblast Growth Factor (FGF) 2 and Insulin-like Growth Factor (IGF) 2 (Song *et al*., 1997; Cheng *et al*., 2008) . In certain types of cancers, glypican-3 was shown to stimulate growth of tumour cells by regulating autocrine/paracrine canonical Wnt signalling. Regulation of migration, adhesion, and actin cytoskeleton organization in mammary tumour cells through Wnt signalling modulation by glypican-3 expression was reported (Stigliano *et al*., 2009). Therefore, the impact of GPC3 expression on other molecules functions is interesting to be investigated.

## 5. Conclusion

Our current investigation revealed for the first time expression of glypican-3 in metastatic neuroblastoma. Despite weak glypican-3 expression, glypican-3 was more predominantly detected in patients with stage 4s of disease and indicated unfavourable disease prognosis. While the present study is the only one exploring the glypican-3 expression in metastatic neuroblastoma specimens, more preliminary data are required on

the potential use of glypican-3 for therapeutic and diagnostic purposes.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of the present paper.

## References


Akutsu, N., H. Yamamoto, S. Sasaki, H. Taniguchi, Y. Arimura, K. Imai and Y. Shinomura.2010. Association of glypican-3 expression with growth signaling molecules in hepatocellular carcinoma. *World J Gastroenterol* **16**(28): 3521-8.

Aviel-Ronen, S., S. K. Lau, M. Pintilie, D. Lau, N. Liu, M. S. Tsao and S. Jothy.2008. Glypican-3 is overexpressed in lung squamous cell carcinoma, but not in adenocarcinoma. *Mod Pathol* **21**(7): 817-25.

Capurro, M., I. R. Wanless, M. Sherman, G. Deboer, W. Shi, E. Miyoshi and J. Filmus.2003. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* **125**(1): 89-97.

Chan, E. S., B. R. Pawel, D. A. Corao, S. Venneti, P. Russo, M. Santi and L. M. Sullivan.2013. Immunohistochemical expression of glypican-3 in pediatric tumors: an analysis of 414 cases. *Pediatr Dev Pathol* **16**(4): 272-7.

Cheng, W., C. J. Tseng, T. T. Lin, I. Cheng, H. W. Pan, H. C. Hsu and Y. M. Lee.2008. Glypican-3-mediated oncogenesis involves the Insulin-like growth factor-signaling pathway. *Carcinogenesis* **29**(7): 1319-26.

Cottereau, E., I. Mortemousque, M. P. Moizard, L. Burglen, D. Lacombe, B. Gilbert-Dussardier, S. Sigaudy, O. Boute, A. David, L. Faivre, J. Amiel, R. Robertson, F. Viana Ramos, E. Bieth, S. Odent, B. Demeer, M. Mathieu, D. Gaillard, L. Van Maldergem, G. Baujat, I. Maystadt, D. Heron, A. Verloes, N. Philip, V. Cormier-Daire, M. F. Froute, L. Pinson, P. Blanchet, P. Sarda, M. Willems, A. Jacquinet, I. Ratbi, J. Van Den Ende, M. Lackmy-Port Lis, A. Goldenberg, D. Bonneau, S. Rossignol and A. Toutain .2013.Phenotypic spectrum of Simpson-Golabi-Behmel syndrome in a series of 42 cases with a mutation in GPC3 and review of the literature. *Am J Med Genet C Semin Med Genet* **163C**(2): 92-105.

Fico, A., F. Maina and R. Dono.2011. Fine-tuning of cell signaling by glypicans. *Cell Mol Life Sci* **68**(6): 923-9.

Filmus, J.2001. Glypicans in growth control and cancer. *Glycobiology* **11**(3): 19R-23R.

Filmus, J. and M. Capurro.2008. The role of glypican-3 in the regulation of body size and cancer. *Cell Cycle* **7**(18): 2787-90.

Filmus, J., M. Capurro and J. Rast.2008. Glypicans. *Genome Biol* **9**(5): 224.

Filmus, J. and S. B. Selleck.2001. Glypicans: proteoglycans with a surprise. *J Clin Invest* **108**(4): 497-501.

Gluer, S., C. Schelp, R. Gerardy-Schahn and D. von Schweinitz.1998. Polysialylated neural cell adhesion molecule as a marker for differential diagnosis in pediatric tumors. *J Pediatr Surg* **33**(10): 1516-20.

Gluer, S., C. Schelp, N. Madry, D. von Schweinitz, M. Eckhardt and R. Gerardy-Schahn.1998. Serum polysialylated neural cell adhesion molecule in childhood neuroblastoma. *Br J Cancer* **78**(1): 106-10.

Haruyama, Y. and H. Kataoka.2016. Glypican-3 is a prognostic factor and an immunotherapeutic target in hepatocellular carcinoma. *World J Gastroenterol* **22**(1): 275-83.

Hughes-Benzie, R. M., A. G. Hunter, J. E. Allanson and A. E. Mackenzie.1992. Simpson-Golabi-Behmel syndrome associated with renal dysplasia and embryonal tumor: localization of the gene to Xqcen-q21. *Am J Med Genet* **43**(1-2): 428-35.

Humpl, T.1995. Neuroblastoma. *World J Urol* **13**(4): 233-9.

Iglesias, B. V., G. Centeno, H. Pascuccelli, F. Ward, M. G. Peters, J. Filmus, L. Puricelli and E. B. de Kier Joffe.2008. Expression pattern of glypican-3 (GPC3) during human embryonic and fetal development. *Histol Histopathol* **23**(11): 1333-40.

Ishola, T. A. and D. H. Chung.2007. Neuroblastoma. *Surg Oncol* **16**(3): 149-56.

Kinoshita, Y., S. Tanaka, R. Souzaki, K. Miyoshi, K. Kohashi, Y. Oda, T. Nakatsura and T. Taguchi.2015. Glypican 3 expression in pediatric malignant solid tumors. *Eur J Pediatr Surg* **25**(1): 138-44.

Lin, H., R. Huber, D. Schlessinger and P. J. Morin.1999. Frequent silencing of the GPC3 gene in ovarian cancer cell lines. *Cancer Res* **59**(4): 807-10.

Man, X. B., L. Tang, B. H. Zhang, S. J. Li, X. H. Qiu, M. C. Wu and H. Y. Wang.2005. Upregulation of Glypican-3 expression in hepatocellular carcinoma but downregulation in cholangiocarcinoma indicates its differential diagnosis value in primary liver cancers. *Liver Int* **25**(5): 962-6.

Maris, J. M., M. D. Hogarty, R. Bagatell and S. L. Cohn.2007. Neuroblastoma. *Lancet* **369**(9579): 2106-20.

Murthy, S. S., T. Shen, A. De Rienzo, W. C. Lee, P. C. Ferriola, S. C. Jhanwar, B. T. Mossman, J. Filmus and J. R. Testa.2000. Expression of GPC3, an X-linked recessive overgrowth gene, is silenced in malignant mesothelioma. *Oncogene* **19**(3): 410-6.

Nakatsura, T., T. Kageshita, S. Ito, K. Wakamatsu, M. Monji, Y. Ikuta, S. Senju, T. Ono and Y. Nishimura.2004. Identification of glypican-3 as a novel tumor marker for melanoma. *Clin Cancer Res* **10**(19): 6612-21.

Nakatsura, T., Y. Yoshitake, S. Senju, M. Monji, H. Komori, Y. Motomura, S. Hosaka, T. Beppu, T. Ishiko, H. Kamohara, H. Ashihara, T. Katagiri, Y. Furukawa, S. Fujiyama, M. Ogawa, Y. Nakamura and Y. Nishimura.2003. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* **306**(1): 16-25.

Ota, S., M. Hishinuma, N. Yamauchi, A. Goto, T. Morikawa, T. Fujimura, T. Kitamura, T. Kodama, H. Aburatani and M. Fukayama.2006. Oncofetal protein glypican-3 in testicular germ-cell tumor. *Virchows Arch* **449**(3): 308-14.

Park, J. R., A. Eggert and H. Caron.2008. Neuroblastoma: biology, prognosis, and treatment. *Pediatr Clin North Am* **55**(1): 97-120, x.

Peters, M. G., E. Farias, L. Colombo, J. Filmus, L. Puricelli and E. Bal de Kier Joffe.2003. Inhibition of invasion and metastasis by glypican-3 in a syngeneic breast cancer model. *Breast Cancer Res Treat* **80**(2): 221-32.

Pilia, G., R. M. Hughes-Benzie, A. MacKenzie, P. Baybayan, E. Y. Chen, R. Huber, G. Neri, A. Cao, A. Forabosco and D. Schlessinger.1996. Mutations in GPC3, a glypican gene, cause the Simpson-Golabi-Behmel overgrowth syndrome. *Nat Genet* **12**(3): 241-7.

Saikali, Z. and D. Sinnett.2000. Expression of glypican 3 (GPC3) in embryonal tumors. *Int J Cancer* **89**(5): 418-22.

Song, H. H., W. Shi and J. Filmus.1997. OCI-5/rat glypican-3 binds to fibroblast growth factor-2 but not to insulin-like growth factor-2. *J Biol Chem* **272**(12): 7574-7.

Stigliano, I., L. Puricelli, J. Filmus, M. C. Sogayar, E. Bal de Kier Joffe and M. G. Peters.2009. Glypican-3 regulates migration, adhesion and actin cytoskeleton organization in mammary tumor cells through Wnt signaling modulation. *Breast Cancer Res Treat* **114**(2): 251-62.

Thway, K., J. Selfe, E. Missiaglia, C. Fisher and J. Shipley.2011. Glypican-3 is expressed in rhabdomyosarcomas but not adult spindle cell and pleomorphic sarcomas. *J Clin Pathol* **64**(7): 587-91.

Tretiakova, M., D. L. Zynger, C. Luan, N. K. Andeen, L. S. Finn, M. Kocherginsky, B. T. Teh and X. J. Yang.2015. Glypican 3 overexpression in primary and metastatic Wilms tumors. *Virchows Arch* **466**(1): 67-76.

Wang, H. L., F. Anatelli, Q. J. Zhai, B. Adley, S. T. Chuang and X. J. Yang.2008. Glypican-3 as a useful diagnostic marker that distinguishes hepatocellular carcinoma from benign hepatocellular mass lesions. *Arch Pathol Lab Med* **132**(11): 1723-8.

Wang, S. K., D. L. Zynger, O. Hes and X. J. Yang.2014. Discovery and diagnostic value of a novel oncofetal protein: glypican 3. *Adv Anat Pathol* **21**(6): 450-60.

Zhang, L., H. Liu, L. Sun, N. Li, H. Ding and J. Zheng.2012. Glypican-3 as a potential differential diagnosis marker for hepatocellular carcinoma: a tissue microarray-based study. *Acta Histochem* **114**(6): 547-52.

Zhu, Z. W., H. Friess, L. Wang, M. Abou-Shady, A. Zimmermann, A. D. Lander, M. Korc, J. Kleeff and M. W. Buchler.2001. Enhanced glypican-3 expression differentiates the majority of hepatocellular carcinomas from benign hepatic disorders. *Gut* **48**(4): 558-64.

Zynger, D. L., M. J. Everton, N. D. Dimov, P. M. Chou and X. J. Yang.2008. Expression of glypican 3 in ovarian and extragonadal germ cell tumors. *Am J Clin Pathol* **130**(2): 224-30.

Zynger, D. L., A. Gupta, C. Luan, P. M. Chou, G. Y. Yang and X. J. Yang.2008. Expression of glypican 3 in hepatoblastoma: an immunohistochemical study of 65 cases. *Hum Pathol* **39**(2): 224-30.