Epigenetic Silencing of $\beta$-Spectrin, a TGF-$\beta$ Signaling/Scaffolding Protein in a Human Cancer Stem Cell Disorder

BECKWITH-WIEDEMANN SYNDROME*\(^2\)

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Hereditary cancer syndromes provide powerful insights into dysfunctional signaling pathways that lead to sporadic cancers. Beckwith-Wiedemann syndrome (BWS) is a hereditary human cancer stem cell syndrome currently linked to deregulated imprinting at chromosome 11p15 and uniparental disomy. However, causal molecular defects and genetic models have remained elusive to date in the majority of cases. The non-pleckstrin homology domain $\beta$-spectrin (b2SP) (the official name for human is Spectrin beta, nonerythrocytic 1 (SPTBN1), isoform 2; the official name for mouse is Spectrin beta 2 (Spnb2), isoform 2), a scaffolding protein, functions as a potent TGF-$\beta$ signaling molecule, member adaptor in tumor suppression and development. Yet, the role of the b2SP in human tumor syndromes remains unclear. Here, we report that b2SP+/− mice are born with many phenotypic characteristics observed in BWS patients, suggesting that b2SP mutant mice phenocopy BWS, and b2SP loss could be one of the mechanisms associated with BWS. Our results also suggest that epigenetic silencing of b2SP is a new potential causal factor in human BWS patients. Furthermore, b2SP+/− mice provide an important animal model for BWS, as well as sporadic cancers associated with it, including lethal gastrointestinal and pancreatic cancer. Thus, these studies could lead to further insight into defects generated by dysfunctional stem cells and identification of new treatment strategies and functional markers for the early detection of these lethal cancers that otherwise cannot be detected at an early stage.

Hereditary cancer syndromes provide powerful insights into our understanding of somatic mutations present in sporadic cancers, as well as implicated cell signaling pathways (1–5).

One clear example is the identification of germ line, inactivating mutations in the adenomatous polyposis coli gene, that encodes a 300-kDa wnt pathway adaptor protein (4). Although germ line mutations in adenomatous polyposis coli are responsible for familial adenomatous polyposis, a rare condition affecting about 1 in 7000 individuals in the United States, somatic mutations in the adenomatous polyposis coli gene are present in more than 70% of colon adenomatous polyps and carcinomas (5). Beckwith-Wiedemann syndrome (BWS)\(^2\) is a hereditary stem cell cancer syndrome currently linked to deregulation of an imprinted cluster on human chromosome 11p15 (6–8). Yet, causal molecular defects and genetic models of this overgrowth syndrome have remained elusive to date in many cases. BWS is associated with an 800-fold increased risk of embryonal neoplasms of childhood and to a lesser extent, hepatocellular carcinoma and renal cell carcinomas. 85% of BWS cases are sporadic, whereas 15% are familial and exhibit an autosomal dominant pattern of inheritance with linkage to chromosome 11p15 (9). Tumor risk estimates vary between 4 and 21% in affected individuals (9). BWS has an incidence of 1/6,000–10,000 births in the United States and a prevalence of 0.07/1,000 births (10). Surprisingly, a 4–9-fold increase in incidence has been recently observed in offspring that result from in vitro fertilization (11, 12).

Molecular defects underlying BWS are only partially understood. Several lines of study suggest that different or sometimes overlapping molecular errors may play a causative role in this disease. Paternal uniparental disomy (UPD, where both homologs of a chromosome pair are inherited exclusively from one parent, resulting in either overexpression or absence of a parent-specific transcript) and loss of imprinting at the insulin-like growth factor 2 (IGF2) gene locus on chromosome 11 associated with overexpression of IGF2, occur in 20 and 10% of

\(^{2}\)The abbreviations used are: BWS, Beckwith-Wiedemann syndrome; SPTBN1, Spectrin beta, nonerythrocytic 1 (Hom sapiens, Gene ID: 6711, OMIM ID 182790); Spnb2, Spectrin beta 2 (Mus musculus, Gene ID: 20742, MIM ID 182790); b2SP, isoform 2 of SPTBN1 or Spnb2, UPD, uniparental disomy.
cases, respectively. Moreover, decreased expression from mutations of cyclin-dependent kinase inhibitor 1C (CDKN1C or p57kip2) or from loss of maternal methylation of potassium voltage-gated channel (KCNO1, previously known as KvLQT1) overlapping transcript 1 (KCNQ1OT1), a non-protein-coding antisense RNA that regulates CDKN1C imprinting has also been reported (8, 13, 14). Furthermore, germ line mutations (homozygous frameshift) of NLRP2 (a member of the Nucleotide-binding oligomerization domain, Leucine-rich Repeat and Pyrin domain family) are also associated with BWS (15). In addition, the transcriptional insulator CCCTC-binding factor, a highly conserved zinc finger protein, has been implicated in BWS and has diverse regulatory functions, including transcriptional activation/repression, insulation, imprinting, and X chromosome inactivation (16, 17). CCCTC-binding factor interacts with its own chromodomain helicase DNA-binding protein 8, forming “active chromatin hubs” mediating long range chromatin interactions between multiple loci such as the IGFl/H19 gene locus which is associated with BWS (8, 18).

Despite these advances in the field, no clear mouse model with BWS to date. Human BWS Tissues and Primary Human BWS Cell Lines—Seven BWS tissues from BWS patients were diagnosed based upon three major criteria (8) (supplemental Fig. 1). Seven human BWS cell lines were developed by Dr. Weksberg (Ontario, Canada) with institutional review board and research ethics approval. Cells were cultured in MEM-$\alpha$ with 10% FBS. The cell lines were named for the molecular abnormality identified (UPD, KvDMR-loss of methylation in KCNO1T1 region or CDKN1C-mutation; Plus (“+”) shows molecular defect, and minus (“−”) shows absence of defect). In addition, cell lines were given a tumor (T) designation if the patient had a tumor or a “no tumor” (NT) designation if no tumor has been detected. Cell lines used in this study were: CDKN1C+NT (referred to as BWSC-1); KvDMR-NT (referred to as BWSC-2); KvDMR+T, hepatoblastoma (referred as BWSC-3); UPD+NT (referred to as BWSC-4); UPD+T, hepatoblastoma (referred to as BWSC-5); two tongue tissue (UPD+) cell lines derived from the same case with biopsies at separate sites (8369F referred to as BWSC-6 and 8370F referred to as BWSC-7). BWSC-2 and BWSC-3 were derived from normal monozygotic twin (absence of KvDMR molecular defect but it had some clinical signs of BWS) and BWS monozygotic twin with KvDMR molecular defect, respectively. Normal human hepatocytes were from the Liver Tissue Cell Distribution System, University of Pittsburgh and University of Minnesota.

Plasmids and Antibodies—The cDNA sequence of β2SP was amplified by gene-specific primers and inserted into pcDNA3.1/V5-His-TOPO (Invitrogen) for transfection studies. Rabbit anti-β2SP-N is an epitope-specific antibody recognizing the β2SP N terminus (36 amino acids). The mouse growth hormone (GH) ELISA kit was from Millipore.

Bisulfite Sequencing and DNA Methylation Analysis—Genomic DNA was bisulfite-modified with an EpiTect Bisulfite kit (Qiagen) according to the manufacturer’s protocols. Prediction of CpG islands in the β2SP promoter and primer design for methylation-specific PCR used urogen software. DNA methylation analysis was by a web tool from RIKEN. Primer pairs (Pmathrm-1) used for BWS cell lines and BWS nontumor tissues methylation-specific PCR and bisulfite sequencing were methylated forward, 5′-CGG TGT TTT TAT AAA TTT TTG CGT C-3′ and reverse, 5′-AAT TCC ATT ATA CCC GAC GTA AGC C-3′; and unmethylated forward, 5′-TTG GTT TTT TTA ATT TTT TTG GTG A-3′ and reverse, 5′-CAA TTC CAT TAT ACC CAA CAT AAC ACC C-3′. Primer Pairs (Pmathrm-2) used for formaldehyde-fixed paraffin-embedded BWS tumors tissues were methylated forward, 5′-TAG TGT TGT TTT TTG GGA AGG CAT TAT C-3′ and unmethylated forward, 5′-TAT GAA AAT AAT ATT AAA AAC ATC TCG C-3′ and unmethylated forward, 5′-GTA GTT TTG TTT GGG AGT TTA TTG-3′ and reverse, 5′-TAA TAT TTT CAA AAA CCA CTC ACC-3′.

Generation of B2SP+/− Mouse and Genotype Analysis—A total of 29 β2SP+/− mice were generated from β2SP+/+ mice intercrossed with β2SP+/- mice. Genotypes were determined by Southern blotting or PCR. All animal procedures were approved by the Institutional Animal Care and Use Committee of Georgetown University Medical Center, Washington, D. C.

Statistics—Statistical analysis was performed by one-way analysis of variance (ANOVA) and unpaired Student’s t test using the INSTAT 3.00 package (GraphPad, San Diego, CA).

RESULTS

Phenotype and Cancer Development in β2SP+/− Heterozygote Mice—Mice with homozygous deletion of β2SP (β2SP−/−) die during midgestation (21). Analysis of 29 β2SP+/− heterozygous mice revealed a 25% increase (35.65 ± 5.72g versus 44.55 ± 8.30g; p < 0.01) in the average body size and mass compared with wild type mice. β2SP+/− heterozygous mice liver increased 23% (1.32 ± 0.19g versus 1.62 ± 0.29, p < 0.1). This was accompanied by macrocrosis, hyperplasia, multiple ear folds, frontal balding, increased incidence of sudden death in the male mutant mice, visceromegaly with multilobed livers, cardiomegaly, renal hypertrophy, and testicular enlargement.
Beckwith-Wiedemann Syndrome, Spectrin β, TGF-β, and Gl Cancer

FIGURE 1. Wild type versus β2SP+/− BWS-like phenotype. A–D, gross comparison of wild type (A, left) versus mutant β2SP+/− (A, right) mouse, wild type (B, left) versus β2SP+/− (B, right) tongue, wild type (C, left) versus β2SP+/− (C, right) kidney, wild type (D, left) versus β2SP+/− (D, right) ear. E, wild type (left) versus β2SP+/− (right) liver. F, wild type (left) versus β2SP+/− (right) spleen. G, wild type (left) versus β2SP+/− (right) stomach. H, wild type (left) versus β2SP+/− (right) lung. I, wild type (left) versus β2SP+/− (right) testis. J, wild type (left) versus β2SP+/− (right) brain. K, wild type (left) versus β2SP+/− (right) heart.

TABLE 1
Comparison of mouse phenotypes with human Beckwith-Wiedemann syndrome

| Manifestation                          | BWS | β2SP+/− | IGF2R−/− | IGF-II overexpression | p57+/− | GPC3+/− |
|----------------------------------------|-----|--------|----------|----------------------|-------|--------|
| Overgrowth                             | +   | +      | +        | −                    | −     | +      |
| Increased circulating IGF-II           | ND  | ND     | +        | −                    | −     | −      |
| Perinatal death                        | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |
| Kidney dysplasia                       | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |
| Thycoma                                | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |
| Breast adenomas                        | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |
| Adrenocortical carcinomas              | +   | +      | +        | −                    | −     | −      |
| Renal cell carcinomas                  | +   | +      | +        | −                    | −     | −      |
| Optic nerve gliomas                    | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |
| Hepatocellular carcinomas              | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |
| Thyroid carcinomas                     | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |
| Pancreatic carcinomas                  | +   | +      | +        | −                    | −     | −      |
| Adrenal cysts                          | +   | +      | +        | −                    | −     | −      |
| Lymphomas                              | +/− | +/−    | +/−      | +/−                  | −/−   | +/−    |

* ND, not studied.
$eta$2SP positive control (HepG2 cells), except for one, UPD+T, hepatoblastoma cells (BWSC-5), where $eta$2SP protein expression is decreased. Our results indicated that a loss (or decrease) of $eta$2SP expression occurs in nearly all BWS patients’ tissues or cell lines examined, no matter what type molecular defects, even in unexplained cases of BWS, such as KvDMR-NT (BWSC-2) cell line, which is derived from a normal monozygotic twin (absence of KvDMR molecular defect, but it had some clinical signs of BWS) and a BWS monozygotic twin with KvDMR molecular defect (Fig. 2 and supplemental Fig. 1).

**$eta$2SP Is Silenced at Its Promoter by DNA Methylation in Human BWS Nontumor/Tumor Tissues and Cell Lines**—DNA methylation patterns are often altered significantly in cancer cells including those from BWS patients. Growing evidence suggests that aberrant DNA methylation of CpG islands around promoter regions can have the same effect as coding region mutations, leading to the inactivation of tumor suppressor genes (24). Because the promoter region of $eta$2SP contains four typical CpG islands (Fig. 3A), we examined their methylation state in genomic DNA isolated from eight cell lines (seven BWS cell lines and the $eta$2SP-positive HepG2 cell line), seven human BWS tissues, and normal human liver tissue utilizing methylation-specific PCR (Fig. 3B) and bisulfite sequencing (Fig. 3C). These results showed a positive correlation between low levels or lack of $eta$2SP expression and methylation in the vicinity of the $eta$2SP promoter in human BWS cells (Fig. 2). In six human BWS cell lines we observed either loss or markedly decreased expression of $eta$2SP (CDKN1c, KvDMR+, UPD+ Tongue-1(UPD) and Tongue-2(UPD)), cytosine residues of CpG dinucleotide in the $eta$2SP promoter region (−1100 to −386) and 5′UTR (−385 to −1) were almost completely methylated, whereas those cytosine residues in two cell lines (HepG2 and UPD−) and normal human liver, which express normal levels of $eta$2SP, were entirely methylation-free (Fig. 3C).

**Reactivation of the $eta$2SP Gene Expression by the DNA Methylation Inhibitor—5′-aza-2′-Deoxycytidine (5′-aza-dC), an inhibitor of DNA methylation, can reactivate gene**

**TABLE 2**

Classification of tumors in $eta$2SP+/− mutant mice

| Categorizes of tumors | Site     | Incidence |
|-----------------------|----------|-----------|
| Hepatocellular carcinoma | Liver | 37.9% (11/29) |
| Hepatoblastoma | NA | NA |
| Adenocarcinoma/lung | Small bowel | 6.9% (2/29) |
| Adenocarcinoma | Lung | 6.9% (2/29) |
| Sarcoma | Abdominal/abdominal mesenchymal | 3.4% (1/29) |
| Clear cell carcinoma | Kidney | 10.3% (3/29) |
| Lymphoma | Spleen | 6.9% (2/29) |
| Adenoma | Breast | 3.4% (1/29) |
| Squamous cell carcinoma | NA | NA |
| Thymoma | NA | NA |
| Carcinoma | Testis | 3.4% (1/29) |
| Glioma | NA | NA |
| Epithelial tumor | Ovary | 3.4% (1/29) |

**FIGURE 2. Loss of $eta$2SP protein expression in BWS.** A, expression of $eta$2SP RNA is decreased greater than −50% in all tested human BWS cells compared with HepG2 cells assessed by quantitative PCR analyses. B, immunohistochemical labeling of $eta$2SP in normal and BWS kidney tumor revealed loss of $eta$2SP expression in BWS kidney tumor compared with normal kidney. C, Western blot analysis human of BWSC demonstrates loss of $eta$2SP expression. D, Western blot analysis of $eta$2SP in human BWST is shown. Results shown in A reflect a mean ± S.E. from three independent experiments, performed in triplicate. ***, $p < 0.001$ compared with control values, determined by t test.
FIGURE 3. DNA methylation pattern of β2SP gene promoter in BWS. A, schematic representation of β2SP promoter and CpG islands. B, methylation status of the β2SP promoter in BWS cell lines and tissues detected by MS-PCR. C, DNA methylation pattern of the β2SP gene promoter in BWS cell lines and tissues identified by bisulfite sequencing. D, methylation status of the β2SP promoter in BWS tumor tissues (BWS/TM) detected by MS-PCR (∙) and bisulfite sequencing (II). Genomic DNA isolation from seven formaldehyde-fixed paraffin-embedded BWS tumor tissues is shown.
expression when hypermethylation of CpG islands is the cause of reduced gene expression (25). To demonstrate regulation of β2SP expression by DNA methylation, three BWS cell lines (two β2SP-negative and one β2SP-positive) were treated with 5′-aza-dC for 6 days. As shown in Fig. 4A, 2.5 μM 5′-aza-dC reactivated β2SP expression in two β2SP-negative BWS cell lines. To confirm this result, we determined DNA methylation levels in β2SP-negative BWS cell line (BWS-3) with 5 μM 5′-aza-dC for 6 days. Results demonstrate significantly decreased methylation levels in both β2SP alleles compared with untreated cells (Fig. 4B). These studies further support the hypothesis that loss of β2SP is a causal event of human BWS.

Increased IGF2 Expression in B2SP+/− Mice Is Similar to That Observed in Human BWS—Increased IGF2 leading to BWS has been reported (8). We performed broad microarray and proteomic analyses on β2SP+/− liver tissues to determine alterations in pathways involved in BWS (supplemental Fig. 2). Microarray profiles of β2SP+/− liver tissues indicated a marked increase in IGF2 expression with minor increased IGFR2 or p57Kip2 levels (supplemental Fig. 2). Immunohistochemical analysis confirmed elevated expression of IGF2 in β2SP+/− liver and pancreatic tissues (Fig. 5A). In contrast, IGF2 receptor is not activated in β2SP+/− pancreatic tissues, except for β2SP+/− liver tissue where a minor increase was noted (Fig. 5AII). p57Kip2 expression levels were slightly increased in β2SP+/− (Fig. 5B, I and II) tissues. However, KCNQ1 expression appears to be increased in β2SP+/− cardiac tissue (Fig. 5B, III and IV).

We next investigated whether increased IGF2 levels in BWS cells could be secondary to loss of repression by β2SP. To determine this, β2SP was ectopically expressed in β2SP-negative BWS cell lines, and levels of IGF2 mRNA were determined. These studies revealed markedly reduced IGF2 mRNA levels (Fig. 5C) in β2SP-transfected BWS cells (a >5-fold reduction). These results indicate that β2SP is required for suppression of IGF2 signaling.

Considering that GH and IGF1 may alter in β2SP+/− mice and may be involved in overgrowth, we measured β2SP+/− the mouse serum GH protein level by ELISA (Fig. 5D) and IGF1 mRNA level by RT-PCR (Fig. 5E). Results show that the levels of GH and IGF1 were not changed in β2SP+/− mice compared with wild type mice. We also measured the mRNA level in BWS patients’ liver by RT-PCR (Fig. 5EII). Results indicated that there are no alterations between normal and BWS, which is consistent with the observation from B2SP+/− mice. These results suggest that loss of β2SP does not affect the GH and IGF1 axis.

**DISCUSSION**

BWS is considered to be linked to a cluster of imprinted genes at human chromosome 11p15.5, including increased IGF2, loss of H19, loss of p57Kip2, and loss of KCNQ1. The human syndrome is characterized by somatic overgrowth, macroglossia, abdominal wall defects, visceromegaly, and an increased susceptibility to a spectrum of tumors that include childhood tumors (26–32). The identification of these genes as well as others including Glypican3 has led to the generation of multiple single/double gene mouse mutants (13, 33–37, 39–43). Yet to date, none of the mouse models develop the full panoply of symptoms and signs of BWS, particularly tumor formation (33). For instance, mutant mice with loss of KCNQ1 develop deafness, abnormalities in inner ear development, mucous neck cell hyperplasia resulting in a 3-fold increase in stomach weight, but no features of BWS, suggesting that
 Increased IGF2 expression in β2SP-/- mice is similar to that observed in human BWS. A, immunohistochemical analysis examining IGF2 (I) and IGF2R (II) in wild type and mutant β2SP liver and pancreas. Increased IGF2 expression in β2SP-/- tissues is shown (arrows). B, p57kip2 expression in wild type (I) and β2SP-/- (II) mouse liver tissues assessed by immunohistochemistry. p57kip2 expression increased in β2SP-/- liver tissues compared with wild type. Potassium voltage-gated channel (KCNQ1) expression in wild type (III) and β2SP-/- (IV) mouse heart tissues was assessed by immunohistochemistry. KCNQ1 expression increased in β2SP-/- liver tissues compared with wild type. C, BWSC-3 showing a high level of IGF2 RNA by quantitative PCR. IGF2 RNA levels decrease in cells transfected with full-length C
/ H9252
2SP plasmid. D, GH protein level in β2SP-/- mice serum measured by ELISA. E, IGF1 mRNA level in β2SP-/- liver tissues (I) and BWS liver (II). Results shown in C reflect a mean ± S.E. (error bar) from three independent experiments. *** p < 0.001 compared with untreated (control) values determined by t test.

KCNQ1 is not responsible for BWS (34). Mice with a maternally inherited deletion of H19 bear a 27% increase in birth weight, and paternal inheritance of the disruption has no effect (35). Deletion of the maternally inherited IGF2R (a maternally expressed gene in mouse but not man, partially functioning as a scavenger for IGF2, mediating its degradation) allele in mice results in increased free IGF2 available for growth, with a 40% increase in birth weight, cardiac defects, and perinatal lethality (36). H19 and IGF2R double heterozygote mice overexpress 7- and 11-fold greater tissue and serum levels of IGF2 and develop a 2-fold increase in birth weight, embryonic lethality, omphalocele, visceromegaly, adrenal cysts, and cleft palate (37). Similar to H19 and IGF2R double heterozygote mice, IGF2-overexpression transgenic chimeric mice made with transgenic ES cells also leads to development of most of the BWS phenotypes, including prenatal overgrowth, polyhydramnios, fetal and neonatal lethality, disproportionate organ overgrowth such as tongue enlargement and skeletal abnormalities (13). Mice with loss-of-function mutations in p57kip2 (a maternally expressed gene encoding a G1 cyclin-dependent kinase inhibitor) and loss of Igf2 imprinting develop placentomegaly, multissue dysplasia, kidney dysplasia, macroglossia, cleft palate, omphalocele, and polydactylly, but not tumors (39–41). Targeted deletions of the murine homolog of glypican-3 gene (GPC3) encoding an extracellular proteoglycan believed to interact with IGF2 and/or other growth factors during development result in several features common to BWS and Simpson-Golabi-Behmel syndrome, namely overgrowth and cystic kidneys (42, 43). Although the data above clearly support the role for IGF2 as the effector for overgrowth, none of the genes appears to be the sole effector for BWS, particularly tumor formation, suggesting that other key proteins remain to be sought. Our surprising findings indicate that the β2SP mutant mice phenocopy BWS by both a genetic and biochemical basis.

Although genetic and biochemical data support the proposed β2SP protein function in human BWS, the situation in vivo is undoubtedly far more complex. Various types of imprinting gene abnormalities on the short arm of chromosome 11 are involved in the etiology of BWS, particularly in the Igf2 gene region (11p15.5). Our data point to an additional or perhaps related mechanism for deregulation of Igf2 involving β2SP. The resemblance of this mouse model to human BWS is substantial but not perfect. The tumors in human BWS are mostly of embryonal cell types, including nephroblastoma, hepatoblastoma, and pancreaticoblastoma, whereas those in the mouse model are largely carcinomas or other “adult” type neoplasms. Neonatal hypoglycemia is observed in 13% of cases and has not been observed in the mutant mice. These distinctions may at least in part reflect
species differences, or they may reflect the difficulties in discerning between embryonal and adult tumors in rodents, such as hepatoblastomas and hepatocellular carcinomas, and in part from the surprising discovery of the syndrome through tumors in surviving older mice in our colony.

Importantly, we report for the first time and provide strong evidence that β2SP is epigenetically silenced in individuals with BWS by examining affected nontumor tissues as well as cell lines. In addition, we observe silencing of β2SP in tissues from affected BWS patients where no loss of imprinting at the IGF2 locus is seen, further supporting the theory that loss of β2SP is one of the causal factors leading to the syndrome. Thus, mutations of β2SP in cancers could lead to new diagnostic, prognostic insights, and new therapeutic strategies into difficult to manage subsets of colorectal and hepatocellular cancers (1, 2). It is possible that in BWS, as in sporadic hepatocellular cancer, derangement in TGF-β signaling in progenitor cells contributes to malignant transformation and eventual cancer development (2, 3). Future studies on genomic imprinting as an epigenetic mechanism controlling parental-origin-specific gene expression, and its perturbations should yield new therapeutics for cancers. Indeed insights into consequences of epigenotype switches at birth, and in BWS from β2SP as a non-pleckstrin homology domain β-spectrin and its scaffolding function may be of profound significance.

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