The role of CpG islands hypomethylation and abnormal expression of neuronal protein synuclein-γ (SNCG) in ovarian cancer

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Submitted: May 1, 2006 *Accepted: May 12, 2006*

Key words: **ovarian cancer; synuclein; SNCG expression; methylation**

Neuroendocrinol Lett 2006; **27**(3):381–386 **PMID:** 16816827 NEL270306A08 © Neuroendocrinology Letters **www.nel.edu**

Abstract

BACKGROUND: The synucleins are a family of small cytoplasmic proteins that are expressed predominantly in neurons. Recently, their expression has been found also in many human cancers. **AIM**: To understand the molecular mechanisms underlying the abnormal expression of *SNCG* in malignant ovarian tumors, in this study, we examined the methylation status of a CpG island located in exon 1 of *SNCG* gene in a panel of ovarian malignant tumors to determine if DNA methylation is related to clinical and histological tumor characteristics. **METHODS**: *SNCG* mRNA expression in tumor samples was assessed by RT-PCR. Methylation status of *SNCG* gene was studied using methylation specific PCR (MSP). **RESULTS**: Study group included 43 ovarian carcinoma samples (40 primary and 3 metastatic). The expression of *SNCG* mRNA was detectable in 33 of 43 ovarian cancer cases (76.7%). There were no significant differences in the mRNA expression between serous or nonserous tumors. The presence of *SNCG* mRNA in tumor samples was not correlated with age and menopausal status of patients, also no correlation was found with clinical stage or histological grading of malignant tumors. In 29 of 43 (67.4%) cases of tumors unmethylated product of MSP amplification was present. In a group of *SNCG* mRNA-positive tumors there were 75.7% (25 of 33) cases with demethylated or hypomethylated exon 1 of SNCG. The differences between the groups were statistically significant (χ^2 =4.46; p=0.034). Demethylation of *SNCG* was not related to clinical tumor stage (p>0.05), but it was strongly associated with tumor grading (χ^2=6.66; p=0.035). Aberrant methylation of *SNCG* was more often seen in tumors of women after menopause (78.2% vs 55%). In postmenopausal women 18 of 33 (62.1%) tumor samples synuclein-γ mRNA expression was found, however the differences were not statistically significant. No correlations between *SNCG* hypomethylation and patient age, clinical stage and tumor grading were found. In 9 of 43 samples (21%) both products of amplification with methylated or unmethylated primer sets were found. In all of these cases *SNCG mRNA* was present. **CONCLUSIONS**: *SNCG* mRNA is expressed in a substantial proportion of malignant ovarian tumors and demethylation is an important event in abnormal synuclein-γ expression in most of these cases.

ORIGINAL ARTICLE

Introduction

Small and highly soluble proteins called synucleins that are predominantly expressed in normal neurons have also been found in various malignant tumors [8]. To date, three synuclein genes coding proteins called synuclein-α, synuclein-β and synuclein-γ have been identified [14]. Expression of first two variants was documented mainly in the central nervous system with both synucleins localized in presynaptic terminals. These proteins are to play an important in the etiology and pathogenesis of several neurodegenerative disorders, such as Alzheimer disease and Parkinson disease [12, 20]. The third member of the neuronal protein family of synucleins, synuclein-γ (SNCG) is a cytoplasmic protein that was found to be localized in the perinuclear area [13]. In several types of human interphase cells it was found in centrosomes [18].

Low levels of SNCG expression were found in the human brain and peripheral nervous system, including dorsal root ganglia and trigeminal ganglia [13,22]. However, SNCG was first identified in a high-throughput direct differential-cDNA-sequencing screen for markers of breast cancer and therefore called "Breast Cancer Specific Gene-1" (BCSG1) [9]. Later studies indicated that outside the nervous system SNCG may be abnormally expressed in a high percentage of tumor tissues of various types of malignant tumors. These neoplasms included ovarian, liver, esophagus, colon, gastric, lung, prostate, cervical, and breast cancer. Interestingly, SNCG was rarely expressed in tumor-matched nonneoplastic adjacent tissues [3,5,16]. The normal cellular function of SNCG is not well understood, but exogenous expression of the protein increases the motility of tumor cells. It has been suggested that SNCG may be involved in modulating the cytoskeleton functions and indirectly promote invasion and spread of tumor cells [3,7]

The mechanism for aberrant expression of synuclein remains unclear. To date, no tumor-specific mutations were found in the protein coding regions of synuclein-γ mRNA in breast tumors and tumor cell lines. Ninkina et al. [1998] suggested that the malignant phenotype could be correlated with overexpression of the wild-type synuclein-γ protein [18]. It has been also proposed that epigenetic alteration of the gene could be a possible mechanism of this process [17]. Down-regulation of synuclein-γ may be related to the molecular mechanism of aberrant methylation in CpG islands [5]. Widschwendter et al. [2004] suggested that failure to repress genes appropriately by abnormal demethylation or hypomethylation of tissue-restricted genes could result in the loss of tissue specificity, overexpression of the proto-oncogenes and could promote cancer development [23]. On the other hand, hypermethylation of normally unmethylated tumor suppressor genes is correlated with a loss of expression in cancer cell lines and primary tumors [1,2]. Strichman-Almashanu et al. have identified unique CpG islands that are methylated specifically in normal tissues and not in cancers [21]. However, compared with numerous tumor suppressor genes that are silenced in malignant tumors, only several genes have been shown to be transcriptionally reactivated by DNA demethylation in cancer cells [23].

The initial observations suggesting that demethylation of SNCG could play a causative role for the expression of this protein cell culture came from an analysis performed by Lu et al.[17]. These investigators have suggested that the exon 1 region of SNCG gene contains a CpG island that is unmethylated in synuclein-γ-positive cancer cell lines SKBR-3 and T47D but densely methylated in synuclein-gamma-negative MCF-7 cells. In the present study we decided to determine if demethylation of the exon 1 is a common molecular determinant responsible for the abnormal expression of SNCG and also to examine the methylation status of exon 1 of SNCG gene in ovarian cancer samples.

Materials and methods

The studied group included 43 women with FIGO stage I through IV ovarian cancers. Tumor tissue was collected from patients who were operated on in the 1st Department of Gynecology of the Medical University in Lublin between 2004–2005. The samples of neoplastic tissue were immediately snap-frozen in liquid nitrogen and stored in -80^0C until further processing. All specimens underwent histological examination to confirm the diagnosis of ovarian malignant tumor. There were 23 serous, 15 mucinous, 2 endometrioid, 2 clear cell, 2 undifferentiated and 3 metastatic cancers. The proportion of malignant cells in all tumor tissue sections used in this study was more than 50%. The histological type and grade of the tumors were classified according to the criteria of the World Health Organization (WHO). The clinical stage of each cancer was established according to International Federation of Gynecology and Obstetrics criteria. The study was approved by the Medical University in Lublin Bioethical Committee. All participants were informed of the nature of the study and gave their written informed consent to participate..

Detection of SNCG methylation by using MSP (methylation specific PCR)

Genomic DNA from tumor tissue samples was extracted with the use of DNAeasy Qiamp Mini Kit (Qiagen,USA) according to manufacturer's protocol. Two μg's of genomic DNA from each sample were modified by sodium bisulfite treatment. The reaction converts all of the unmethylated cytosines to uracils, whereas methylated cytosines remain unchanged. For DNA modification a CpG Modification Kit (Chemicon, USA) was used according to manufacturer's instruction. To identify a possible correlation between DNA methylation of exon 1 and expression of SNCG, we examined the methylation status of the DNA obtained from tumor samples by MSP method described by Herman et al. [6]. Two sets of specific primers designed to distinguish methylated DNA from unmethylated DNA in the promoter regions

Table I. Sequence of PCR primers used for MSP reaction.

Primer		Sequence 5′-3′	Size, bp	Anneal. temp.
SNCGM	sense	TCGTATTAATATTTTATCGGCGT	102	52°C
	antisense	ACGAAACTAAATCTCCCTACGAACTACGT		
SNCGU	sense	GGTTTTTGTATTAATATTTTATTGGTG	102	52°C
	antisense	ACAAAACTAAATCTCCCTACAAACTACAA		
RTSNCG	sense	ATGGATGTCTTCAAGAAGGG	384	55°C
	antisense	CTAGTCTCCCCCACTCTGGG		
RTACTIN	sense	GCTGTGCTATCCCTGTACGC	314	55°C
	antisense	TGCCTCAGGGCAGCGGAACC		

Table II. Clinical and histological characteristics of studied tumors.

Histological type	Number of patiens	Mean patients' age (range)	Grading			FIGO clinical stage	
			G1	G2	G3	I/II	III/IV
serous	16	57 (24-72)	1	8	7	1	15
mucinous	4	64 (41-75)	0	3	1	0	4
endometrioid	1	45 (43-47)	0	1	0	0	1
clear cell	3	65 (61-70)	0	2	1	2	1
undifferentiated	4	57 (53-60)	0	2	2	0	4
metastatic	6	58 (54-65)	0	2	6	0	8
other	7	49 (41-55)	1	0	5	2	4

of the SNCG gene were used. Primers were designed to amplify the same region of exon 1 from bp –139 to –37, covering the CpG sites 2–11 and yielding a product of 102 bp. The primer's sequences and annealing temperature are shown in Table I. The PCR mixture contained 2.5 µl of 10x PCR buffer (Qiagen); 5µl 5xQ-Solution(Qiagen) 200 µM dNTPs; primers (0.5 µM each per reaction) and bisulfite-modified DNA (30 ng) or unmodified DNA (30–50 ng) used as a template, all in a final volume of 25µl. This reaction mix was amplified with Qiagen Taq polymerase (Qiagen). Water blanks without added DNA were included and used as negative PCR controls in each assay. The PCR conditions for both sets of primers were as follows: first cycle at 94°C for 1 min to activate the hot start enzyme, then 30 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 30 s, and a final elongation at 72°C for 5 min. PCR products were analyzed on 2% agarose gels containing ethidium bromide.

Detection of SNCG- mRNA expression by using RT-PCR

RNA Isolation. Tissue samples were homogenized in 1 ml of Trizol Reagent (Life Technologies, Inc.; 1 ml/35–45 mg of tissue). The homogenized samples were incubated for 5 min at room temperature. After addition of 0.2 ml of chloroform, the samples were vigorously shaken for 15 s and incubated at room temperature for another 3 min. The samples were then spun at 13,500 rpm for 15 min at 4°C. Following centrifugation, the aqueous phase containing total RNA was transferred to a fresh tube and the same volume of isopropyl alcohol was added to the tube. The samples were incubated for 10 mins at room temperature and RNA was precipitated by centrifugation. Next, RNA pellets were washed with 1 ml of 75% ethanol and dissolved in 40 µl of RNase-free water.

RT-PCR Analyses. RT-PCR analysis was performed with the use of a standard RT-PCR with the primers specific for human SNCG and beta-actin. Three µg of total RNAs were used for reverse transcription reaction using oligo(dT) primers. The samples were heated to 70°C for 10 min and then quickly chilled on ice. Then, 4 µl of 5X first strand buffer, 2µl of 0.1 M DTT, 1 µl of 10 mM deoxynucleoside triphosphate, and 200 units of reverse transcriptase were added to the samples. The mixtures were incubated at 42°C for 60 min, and one-fifth of this reaction was amplified by PCR using PCR kit. Each reaction consisted of 30 cycles in the GeneAmp PCR System 2400 (Perkin-Elmer). The parameters for PCR were: denaturation at 95°C for 30 s; annealing at 55°C for 30 s; and elongation at 72°C for 60 s. One-third of the PCR products were electrophoresed through 1% agarose gel. Integrity and loading control of the RNA samples were ascertained by documenting actin expression with a set of primers for 314-bp beta-actin. The primer's sequences and annealing temperature are shown in Table I.

Statistical Analysis. All data were tested by χ^2 test performed with the use of Statistica ver. 6.0 (Statsoft,USA) with $p<0.05$ considered to be significant.

Table III. Detection of methylated and unmethylated CpG islands by MSP method and *SNCG*mRNA expression in malignant ovarian tumors related to selected clinical and histological parameters.

	Methylated SNCG	Demethylated SNCG	SNCGmRNA
		Number of patients	
	23(39.53%)	25(58.14%)	33(76.74%)
		Menopausal status (MNP)	
Before MNP	13(65.0%)	11(55.0%)	16(48.48%)
After MNP	10(43.48%)	18(78.26%)	17(51.52%)
		Histological Grading	
G1	2(100%)	0(0.00%)	2(6.06%)
G2	9(50.0%)	15(83.33%)	13(39.39%)
G3	12(52.17%)	14(60.87%)	18(54.55%)
		FIGO stage	
I and II	2(40%)	4(80.0%)	4(80.0%)
III and IV	21(55.26%)	25(65.79%)	29(76.32%)
		Histological type	
serous	8(50%)	11(68.75%)	12(75%)
nonserous	15(55.6%)	18(66.67%)	21(77.7%)

Results

Clinical and histological characteristics of patients and their tumor samples used in this study are listed in Table II. The expression of SNCG mRNA was detectable in 33 of 43 ovarian cancer cases (76.7%). There were no significant differences in the mRNA expression between serous or nonserous tumors. The presence of SNCG mRNA in tumor samples was not correlated with age and menopausal status of patients, also no correlation was found with clinical stage or histological grading of malignant tumors. Detection rates of SNCG mRNA and percentage of methylated or unmethylated CpG islands in malignant ovarian tumors related to selected clinical and histological parameters are shown in Table III.

We found that in 29 of 43 (67.4%) cases of ovarian cancer unmethylated product of MSP amplification was present. In a group of SNCG mRNA-positive tumors there were 75.7% (25 of 33) cases with demethylated or hypomethylated exon 1 of SNCG and the differences between the groups were statistically significant (χ^2 =4.46; p=0.034). Demethylation of SNCG was not related to clinical tumor stage (p>0.05), but it was correlated with tumor grading χ^2=6.66; p=0.035. Aberrant methylation of SNCG was more often seen in tumors of women after menopause (78.2% vs 55%). In 18 of 33 (62.1%) postmenopausal women tumor samples synuclein-γ expression was found, however the difference was not statistically significant. No correlation between SNCG hypomethylation in tumor samples with patient age, clinical stage and tumor histology was found. In 9 of 43 samples (21%) both products of amplification with methylated or unmethylated primer sets were found. In all of these cases SNCG mRNA was present.

Discussion

The onset of cancer is associated with the silencing of tumor suppressor genes and activation of proto-oncogenes [2]. Because previous data suggested possible role of demethylation in SNCG gene expression in ovarian and breast cancer cells in culture, we wanted to know whether the demethylation-dependent gene expression occurs under in vivo conditions as well. First, using RT-PCR assays, we examined SNCG mRNA presence in malignant ovarian tumors. We have found that expression of synuclein-γ was evident in 77% of examined cancer cases. There were no significant differences in SNCG expression between serous or nonserous ovarian tumors. Analyzed clinical parameters included patient's age, menopausal status, clinical stage and tumor grading and had no influence on the expression pattern of SNCGmRNA. Our results confirmed previous observations presented by Bruening et al.who have found that expression of synuclein-γ protein was evident in 75% (33 of 45) ovarian cancer samples [3]. In this study 87% of examined ovarian carcinoma cell lines expressed at least one member of the synuclein family (α, β or γ). The authors have not found any correlation between the clinical stage of the ovarian tumor and expression of synuclein-γ. However, highly punctate SNCG expression was found in 20% of preneoplastic lesions of the ovary, suggesting that synuclein-γ up-regulation may occur early in the development of some ovarian carcinomas. Li et al [2004] have found abnormal expression of synuclein-γ in 69% of pancreatic carcinomas. SNCG was detectable by Western blot in serum samples from 21 of 56 patients (38%), suggesting that synuclein-γ may be able to serve as a marker for pancreatic carcinoma if a far more sensitive detection method can be developed [15].

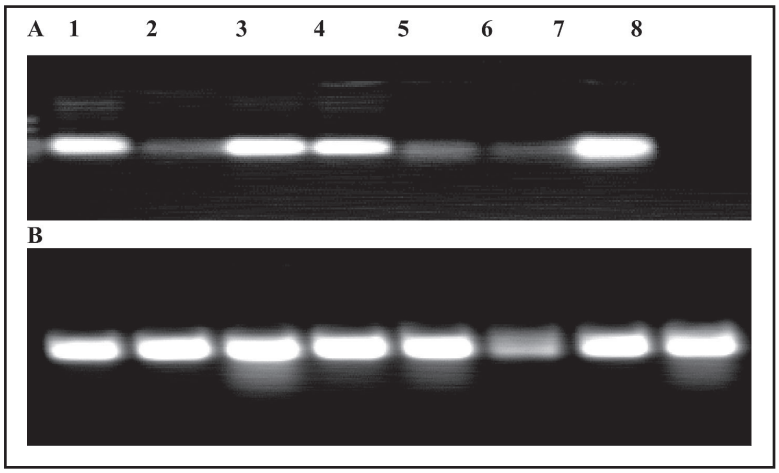

Figure1. Expression of synuclein-γ as assessed by RT-PCR analysis (A). The β-actin gene was used as a control of quantity of total RNA (B). RT-PCR demonstrated the presence or absence *SNCG* mRNA in the following histological type of ovarian cancer samples: serous (Lane 1 and 2), mucinous (Lane 3), endometrioid (Lane 4), clear cell (Lane 5), metastatic (Lane 6 and 7) and undifferentiated (Lane 8)

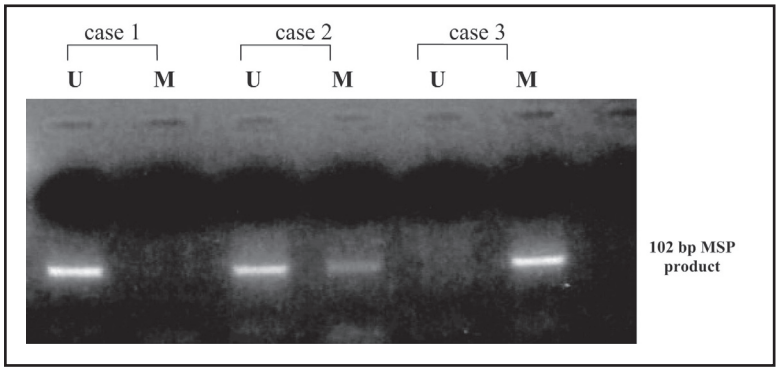

Figure 2. Different methylation status of SNCG CpG islands in three cases of ovarian cancer. Bisulfite-modified genomic DNA was used as template to amplify exon 1of the SNCG. The PCR reactions with primers used to detect methylated DNA and unmethylated DNA were performed separately. M – methylated primers; U – unmethylated primers.

Previous studies have suggested that SNCG could function as an oncogene in cancer cells [10]. The first piece of evidence suggesting a possible role of synuclein-γ in the development of ovarian carcinoma came from an analysis of the expressed sequence tag database [13]. Lavedan et al. found that 37% of the human SNCG sequences were originated from an ovarian tumor library, and the rest of the sequences were from brain and breast tumor libraries, thereby predicting that SNCG may also be overexpressed in ovarian tumor. Pan et al. suggested that abnormal expression of the SNCG activates the survival protein ERK1/2 and blocks the activation of proapoptotic factor called JNK [19]. Therefore synuclein, in its oncogenic form i.e. overexpressed may contribute to tumor development by protecting cancer cells under adverse conditions through modulating the ERK and JNK pathways. The investigators observed that SNCG-expressing cells were significantly more resistant to the chemotherapeutic drugs as compared with the parental cells. Recently, Inaba et al. [2005] suggested that SNCG intracellularly associates with the mitotic checkpoint protein BubR1 together and therefore caused the inhibition of the normal function of BubR1, thereby promoting genetic instability [7].

To determine whether the expression of *SNCG* in ovarian cancer is associated with hypomethylation, we analyzed methylation status of DNA isolated from samples of tumor tissues. It is thought that overexpression of synuclein-γ is connected with the molecular mechanism of aberrant methylation in CpG islands [16]. This process is now considered an important epigenetic alteration occurring in many human cancers [1,2]. In our study detection of *SNCG mRNA* in tumor-positive tumors was strongly associated with demethylation or hypometylation of *SNCG* gene. Methylation status was not correlated with FIGO stage or histological type of tumor. Tumor grading was strongly associated with methylation status but due to relatively small group of studied samples (43 cases) this observation requires further confirmation. Another interesting observation was that in 21% of samples both products of amplification were present and all these cases were *SNCGmRNA*-positive. This observation could suggested that partial methylation of *SNCG* probably does not influence synuclein expression in ovarian cancer tissue. Gupta et al. compared methylation status of *SNCG* and the expression of synuclein-γ in breast and ovarian cancer cells lines [5]. They also observed also a strong correlation of hypomethylation of the CpG island and *SNCG* expression in cancer cell lines. The methylation pattern in ovarian cancer cells was different from that in breast cancer cells. The analyzed CpG sites in ovarian cancer cells were all methylated instead of selective methylation at certain sites shown in breast cancer cells, thereby suggesting a tissue-specific methylation pattern. Authors have found that when exon 1 was partially and heterogeneously methylated *SNCG* expression in breast cancer cells was not detected [5]. Yanagawa et al. exam-

ined the expression and methylation status of *SNCG* in primary gastric cancers, gastric cancer cell lines, and non-neoplastic gastric mucosal tissues [25]. These investigators found that 38% of primary gastric cancers showed apparent *SNCG* demethylation and it was more frequent in primary gastric cancers positive for lymph node metastasis than in cancers without lymph node involvement, and also more common in stage II–IV than in stage I cancers. Our results suggest that members of the synuclein family, SNCG is overexpressed in ovarian cancer due to aberrant methylation process. The function of these small proteins in malignant cells and in other nonneuronal tissues is still unclear. Overexpression of synuclein-γ in breast tumor cells recently has been shown to directly increase the invasiveness of these cells [10,11] As such, any proteins involved in this process may be potential targets for therapies designed to cancer treatments. Additional studies are required to determine if synuclein-γ is expressed in benign and low malignant potential ovarian tumors, and early stage ovarian adenocarcinomas. An elucidation of the reasons for *SNCG* overexpression in ovarian tumors may shed some light on the pathogenesis of cancer progression. Future studies are needed to clearly define the function of this protein in malignant tumor development. Further studies could provide insight to understand the molecular mechanisms that control the methylation status of *SNCG* gene.

In summary, our results suggest that *SNCG* is expressed in a substantial proportion of malignant ovarian tumors and demethylation is an important event in abnormal synuclein-γ expression in most of these cases.

REFERENCES

1 Ahluwalia A, Yan P, Hurteau JA, Bigsby RM, Jung SH, Huang TH, Nephew KP. DNA methylation and ovarian cancer. I. Analysis of CpG island hypermethylation in human ovarian cancer using differential methylation hybridization. Gynecol Oncol 2001; **82**:261–268.

2 Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 1998; **72**:141–196.

3 Bruening W, Giasson BI, Klein-Szanto AJ, Lee VM, Trojanowski JQ, Godwin AK. Synucleins are expressed in the majority of breast and ovarian carcinomas and in preneoplastic lesions of the ovary. Cancer 2000; **88**(9):2154–2163.

4 Clayton DF, George JM. The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. Trends Neurosci 1998; **21**(6):249–54.

5 Gupta A, Godwin AK, Vanderveer L, Lu AP, Liu J. Hypomethylation of the *Synuclein*-Gene CpG island promotes its aberrant expression in breast carcinoma and ovarian carcinoma. Cancer Research 2003; **63**: 664–673.

6 Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Methylation-specific, PCR: a novel PCR assay for methylation status of CpG islands. Proc. Natl Acad Sci USA 1996; **93**(18):9821–9826.

7 Inaba S, Li C, Shi YE, Song DQ, Jiang JD, Liu J. Synuclein gamma inhibits the mitotic checkpoint function and promotes chromosomal instability of breast cancer cells. Breast Cancer Res Treat 2005; **94**(1):25–35.

8 Jakes R, Spillantini M, Goedert M. Identification of two distinct synucleins from human brain. FEBS Lett 1994; **345**(1):27–34.

9 Ji H, Liu YE, Jia T, Wang M, Liu J, Xiao G, Joseph BK, Rosen C, Shi YE: Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing. Cancer Res 1997; **57**(4):759–764.

10 Jia T, Liu Y, Liu J Shi Y. Stimulation of breast cancer invasion and metastasis by breast cancer-specific synuclein (SNCG). Cancer Res 1999; **59**(3)**:**742–747.

11 Jiang Y, Liu YE, Goldberg ID, Shi YE Gamma synuclein, a novel heat-shock protein-associated chaperone, stimulates ligand-dependent estrogen receptor alpha signaling and mammary tumorigenesis. Cancer Res 2004; **64**(13):4539–4546.

12 Kawashima M, Suzuki SO, Doh-ura K, Iwaki T. alpha-Synuclein is expressed in a variety of brain tumors showing neuronal differentiation. Acta Neuropathol (Berl) 2000; **99**(2):154–60.

13 Lavedan C, Leroy E, Dehejia A, et al. Identification, localization and characterization of the human γ-synuclein gene. Hum Genet 1998; **103**:106–112.

14 Lavedan C. The synuclein family. Genome Res. 1998 Sep; **8**(9):871–80.

15 Li Z, Sclabas GM, Peng B, Hess KR, Abbruzzese JL, Evans DB, Chiao PJ.Overexpression of synuclein-gamma in pancreatic adenocarcinoma. Cancer 2004; **101**(1):58–65.

16 Liu H, Liu W, Wu Y, Zhou Y, Xue R, Luo C, Wang L, Zhao W, Jiang JD, Liu J. Loss of epigenetic control of synuclein-gamma gene as a molecular indicator of metastasis in a wide range of human cancers. Cancer Res 2005; **65**(17):7635–7643.

17 Lu A, Gupta A, Li C, Ahlborn TE, Ma Y, Shi EY, Liu J. Molecular mechanisms for aberrant expression of the human breast cancer specific gene 1 in breast cancer cells: control of transcription by DNA methylation and intronic sequences Oncogene 2002; **20**(37):5173–5185.

18 Ninkina NN, Alimova-Kost MV, Paterson JW, Delaney L, Cohen BB, Imreh S, Gnuchev NV, Davies AM, Buchman VL. Organization, expression and polymorphism of the human persyn gene. Hum Mol Genet 1998; **7**(9):1417–1424.

19 Pan ZZ, Bruening W, Giasson BI, Lee VM, Godwin AK. Gamma-synuclein promotes cancer cell survival and inhibits stress- and chemotherapy drug-induced apoptosis by modulating MAPK pathways. J Biol Chem 2002; **277**(38):35050–60.

20 Sopher BL, Koszdin KL, McClain ME, Myrick SB, Martinez RA, Smith AC, La Spada AR.Genomic organization, chromosome location, and expression analysis of mouse beta-synuclein, a candidate for involvement in neurodegeneration. Cytogenet Cell Genet 2001; **93**(1–2):117–23.

21 Strichman-Almashanu LZ, Lee RS, Onyango PO, Perlman E, Flam F, Frieman M B, Feinberg AP. A genome wide screen for normally methylated human CpG islands that can identify novel imprinted genes. Genome Res 2002; **12**(4)*:* 543–554.

22 Surguchov A, Palazzo RE, Surgucheva I. Gamma synuclein: subcellular localization in neuronal and non-neuronal cells and effect on signal transduction. Cell Motil Cytoskeleton 2001; **49**(4):218–228.

23 Widschwendter M, Jiang G, Woods C, Muller HM, Fiegl H, Goebel G, Marth C, Muller-Holzner E, Zeimet AG, Laird PW, Ehrlich M. DNA hypomethylation and ovarian cancer biology. Cancer Res 2004; **64**(13):4472–80.

24 Wu K, Weng Z, Tao Q, Lin G, Wu X, Qian H, Zhang Y, Ding X, Jiang Y, Shi YE. Stage-specific expression of breast cancer-specific gene gamma-synuclein. Cancer Epidemiol Biomarkers Prev 2003; **12**(9):920–5.

25 Yanagawa N, Tamura G, Honda T, Endoh M, Nishizuka S, Motoyama T. Demethylation of the synuclein gamma gene CpG island in primary gastric cancers and gastric cancer cell lines. Clin Cancer Res 2004; **10**(7):2447–51.