breast cancer cell lines, this gene showed an indication of a reciprocal expression with *BRCA1*, with which it shares a bi-directional promoter (Xu et al., 1997b; Suen and Goss, 1999). However, it was decreased in primary breast cancer cell cultures, though much less than the NBR1 1A and 1B isoforms. The reason for the discrepancy between permanent cell lines and short-term cultures is not clear. The possible effect of the cell culture conditions on the expression of the studied genes could be evaluated by re-examination of their mRNA levels in fresh tumour specimens.

It has been known for some time that the expression of *BRCA1* is regulated during the cell cycle, with elevated levels at G1/S transition and during the S phase (Gudas et al., 1996; Vaughn et al., 1996). At present, experiments are in progress in our laboratory to compare the mRNA levels of *NBR2* and *NBR1* genes during the cell cycle of normal and malignant cells, with the idea that a possible temporal deregulation of these genes during the cell cycle could possibly result in decreased expression of the non-mutated copy of *BRCA1* tumour suppressor gene.

## References


Barker, D. F., Liu, X., Almeida, E. R. (1996) The BRCA1 and 1A1.3B promoters are parallel elements of a genomic duplication at 17q21. *Genomics* **38**, 215-222.

Brown, M. A., Xu, C. F., Nicolai, H., Griffiths, B., Chambers, J. A., Black, D., Solomon, E. (1996) The 5' end of the BRCA1 gene lies within a duplicated region of human chromosome 17q21. *Oncogene* **12**, 2507-2513.

Campbell, I. G., Nicolai, H. M., Foulkes, W. D., Senger, G., Stamp, G. W., Allan, G., Boyer, C., Jones, K., Bast, R. C., Solomon, E. (1994) A novel gene encoding a B-box protein within the BRCA1 region at 17q21.1. *Hum. Mol. Genet.* **3**, 589-594.

Catteau, A., Harris, W. H., Xu, C. F.,Solomon, E. (1999a) Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics. *Oncogene* **18**, 1957-1965.

Catteau, A., Xu, C. F., Brown, M. A., Hodgson, S., Greenman, J., Mathew, C. G., Dunning, A. M., Solomon, E. (1999b) Identification of a C/G polymorphism in the promoter region of the BRCA1 gene and its use as a marker for rapid detection of promoter deletions. *Br. J. Cancer* **79**, 759-763.

Chang, E. C., Barr, M., Wang, Y., Jung, V., Xu, H. P., Wigler, M. H. (1994) Cooperative interaction of S. pombe proteins required for mating and morphogenesis. *Cell* **79**, 131-141.

Chapman, M. S., Verma, I. M. (1996) Transcriptional activation by BRCA1. *Nature* **382**, 678-679.

Chomczynski, P., Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156-159.

Cortez, D., Wang, Y., Qin, J., Elledge, S. J. (1999) Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. *Science* **286**, 1162-1166.

Dimitrov, S., Brennerova, M., Forejt, J. (2001) Expression profiles and intergenic structure of head-to-head oriented Brca1 and Nbr1 genes. *Gene* **262**, 89-98.

Dobrovic, A., Simpfendorfer, D. (1997) Methylation of the BRCA1 gene in sporadic breast cancer. *Cancer Res.* **57**, 3347-3350.

Easton, D. F., Bishop, D. T., Ford, D., Crockford, G. P. (1993) Genetic linkage analysis in familial breast and ovarian cancer: results from 214 families. The Breast Cancer Linkage Consortium. *Am. J. Hum. Genet.* **52**, 678-701.

Esteller, M., Silva, J. M., Dominguez, G., Bonilla, F., Matias-Guiu, X., Lerma, E., Bussaglia, E., Prat, J., Harkes, I. C., Repasky, E. A., Gabrielson, E., Schutte, M., Baylin, S. B., Herman, J. G. (2000) Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumours. *J. Natl. Cancer. Inst.* **92**, 564-569.

Fan, S., Wang, J., Yuan, R., Ma, Y., Meng, Q., Erdos, M. R., Pestell, R. G., Yuan, F., Auborn, K. J., Goldberg, I. D., Rosen, E. M. (1999) BRCA1 inhibition of oestrogen receptor signaling in transfected cells. *Science* **284**, 1354-1356.

Futreal, P. A., Liu, Q., Shattuck-Eidens, D., Cochran, C., Harshman, K., Tavtigian, S., Bennett, L. M., Haugen-Strano, A., Swensen, J., Miki, Y., et al. (1994) BRCA1 mutations in primary breast and ovarian carcinomas. *Science* **266**, 120-122.

Gause, W. C., Adamovicz, J. (1995) Use of PCR to quantitate relative differences in gene expression. In: *PCR Primer Laboratory Manual*, eds. Dieffenbach, C. W. and Dveksler, G. S., pp. 293-312. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, USA.

Gudas, J. M., Li, T., Nguyen, H., Jensen, D., Rauscher, F. J., Cowan, K. H. (1996) Cell cycle regulation of BRCA1 messenger RNA in human breast epithelial cells. *Cell Growth Differ.* **7**, 717-723.

Hashizume, R., Fukuda, M., Maeda, I., Nishikawa, H., Oyake, D., Yabuki, Y., Ogata, H., Ohta, T. (2001) The ring heterodimer brca1-bard1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. *J. Biol. Chem.* **276**, 14537-14540.

Hofmann, K., Bucher, P. (1996) The UBA domain: a sequence motif present in multiple enzyme classes of the ubiquitination pathway. *Trends Biochem. Sci.* **21**, 172-173.

Jeffy, B. D., Schultz, E. U., Selmin, O., Gudas, J. M., Bowden, G. T., Romagnolo, D. (1999) Inhibition of BRCA-1 expression by benzo[a]pyrene and its diol epoxide. *Mol. Carcinog.* **26**, 100-118.

Jensen, D. E., Proctor, M., Marquis, S. T., Gardner, H. P., Ha, S. I., Chodosh, L. A., Ishov, A. M., Tommerup, N., Vissing, H., Sekido, Y., Minna, J., Borodovsky, A., Schultz, D. C., Wilkinson, K. D., Maul, G. G., Barlev, N., Berger, S. L., Prendergast, G. C., Rauscher, F. J. (1998) BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. *Oncogene* **16**, 1097-1112.

Magdinier, F., Ribieras, S., Lenoir, G. M., Frappart, L., Dante, R. (1998) Down-regulation of BRCA1 in human sporadic breast cancer; analysis of DNA methylation patterns of the putative promoter region. *Oncogene* **17**, 3169-3176.

Mancini, D. N., Rodenhiser, D. I., Ainsworth, P. J., O'Malley, F. P., Singh, S. M., Xing, W., Archer, T. K. (1998) CpG methylation within the 5' regulatory region of the BRCA1 gene is tumour specific and includes a putative CREB binding site. *Oncogene* **16**, 1161-1169.

Matoušková, E., Dudorkinova, D., Pavlíková, L., Povýšil, C., Veselý, P. (1998) Clonal expansion of epithelial cells from primary human breast carcinoma with 3T3 feeder layer technique. *Folia Biol. (Praha)* **44**, 67-71.

Matoušková, E., Dudorkinova, D., Krásná, L.,Veselý, P. (2000) Temporal in vitro expansion of the luminal lineage of human mammary epithelial cells achieved with the 3T3 feeder layer technique. *Breast Cancer Res. Treat.* **60**, 241-249.

Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P. A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L. M., Ding, W., et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* **266**, 66-71.

Nomura, N., Nagase, T., Miyajima, N., Sazuka, T., Tanaka, A., Sato, S., Seki, N., Kawarabayasi, Y., Ishikawa, K., Tabata, S. (1994) Prediction of the coding sequences of unidentified human genes. II. The coding sequences of 40 new genes (KIAA0041-KIAA0080) deduced by analysis of cDNA clones from human cell line KG-1. *DNA Res.* **1**, 223-229.

Ponting, C. P., Blake, D. J., Davies, K. E., Kendrick-Jones, J., Winder, S. J. (1996) ZZ and TAZ: new putative zinc fingers in dystrophin and other proteins. *Trends Biochem. Sci.* **21**, 11-13.

Rahman, N., Stratton, M. R. (1998) The genetics of breast cancer susceptibility. *Annu. Rev. Genet.* **32**, 95-121.

Ribieras, S., Magdinier, F., Leclerc, D., Lenoir, G., Frappart, L., Dante, R. (1997) Abundance of BRCA1 transcripts in human cancer and lymphoblastoid cell lines carrying BRCA1 germ-line alterations. *Int. J. Cancer* **73**, 715-758.

Ruffner, H., Joazeiro, C. A., Hemmati, D., Hunter, T., Verma, I. M. (2001) Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. *Proc. Natl. Acad. Sci. USA* **98**, 5134-5139.

Ruffner, H., Verma, I. M. (1997) BRCA1 is a cell cycle-regulated nuclear phosphoprotein. *Proc. Natl. Acad. Sci. USA* **94**, 7138-7143.

Scully, R., Ganesan, S., Brown, M., De Caprio, J. A., Cannistra, S. A., Feunteun, J., Schnitt, S., Livingston, D. M. (1996) Location of BRCA1 in human breast and ovarian cancer cells. *Science* **272**, 123-126.

Schneider, S. M., Offterdinger, M., Huber, H., Grunt, T. W. (2000) Activation of retinoic acid receptor alpha is sufficient for full induction of retinoid responses in SK-BR-3 and T47D human breast cancer cells. *Cancer Res.* **60**, 5479-5487.

Schultz, J., Copley, R. R., Doerks, T., Ponting, C. P., Bork, P. (2000) SMART: a web-based tool for the study of genetically mobile domains. *Nucleic Acids Res.* **28**, 231-234.

Sourvinos, G., Spandidos, D. A. (1998) Decreased BRCA1 expression levels may arrest the cell cycle through activation of p53 checkpoint in human sporadic breast tumours. *Biochem. Biophys. Res. Commun.* **245**, 75-80.

Suen, T. C., Goss, P. E. (1999) Transcription of BRCA1 is dependent on the formation of a specific protein-DNA complex on the minimal BRCA1 Bi-directional promoter. *J. Biol. Chem.* **274**, 31297-31304.

Thompson, M. E., Jensen, R. A., Obermiller, P. S., Page, D. L., Holt, J. T. (1995) Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nat. Genet.* **9**, 444-450.

Tibbetts, R. S., Cortez, D., Brumbaugh, K. M., Scully, R., Livingston, D., Elledge, S. J., Abraham, R. T. (2000) Functional interactions between BRCA1 and the checkpoint kinase ATR during genotoxic stress. *Genes Dev.* **14**, 2989-3002.

Vaughn, J. P., Davis, P. L., Jarboe, M. D., Huper, G., Evans, A. C., Wiseman, R. W., Berchuck, A., Iglehart, J. D., Futreal, P. A., Marks, J. R. (1996) BRCA1 expression is induced before DNA synthesis in both normal and tumour-derived breast cells. *Cell Growth Differ.* **7**, 711-715.

Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S. J., Qin, J. (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.* **14**, 927-939.

Welcsh, P. L., King, M.-C. (2001) BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Hum. Mol. Genet.* **10**, 705-713.

Wilson, C. A., Ramos, L., Villasenor, M. R., Anders, K. H., Press, M. F., Clarke, K., Karlan, B., Chen, J. J., Scully, R., Livingston, D., Zuch, R. H., Kanter, M. H., Cohen, S., Calzone, F. J., Slamon, D. J. (1999) Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. *Nat. Genet.* **21**, 236-240.

Xu, C. F., Brown, M. A., Nicolai, H., Chambers, J. A., Griffiths, B. L., Solomon, E. (1997a) Isolation and characterisation of the NBR2 gene which lies head to head with the human BRCA1 gene. *Hum. Mol. Genet.* **6**, 1057-1062.

Xu, C. F., Chambers, J. A., Solomon, E. (1997b) Complex regulation of the BRCA1 gene. *J. Biol. Chem.* **272**, 20994-20997.

Zhong, Q., Chen, C. F., Li, S., Chen, Y., Wang, C. C., Xiao, J., Chen, P. L., Sharp, Z. D., Lee, W. H. (1999) Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. *Science* **285**, 747-750.