Expression of *BRCA1, NBR1* and *NBR2* Genes in Human Breast Cancer Cells

(*NBR1* isoforms / mRNA steady-state levels / semi-quantitative RT PCR / octicosapeptide repeat / ubiquitin-associated domain)

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Abstract. *BRCA1* is a tumour suppressor gene with a caretaker function in the DNA-damage repair and the maintenance of genome integrity. The human *BRCA1* and *NBR2* genes and the homologous Brca1 and Nbr1 mouse genes are situated head-to-head on human chromosome 17q21 and on mouse chromosome 11, respectively. Their transcription start sites, located on opposite DNA strands, are separated by 218 bp in humans, and by 289 bp in mice. Because of this intimate contact and because of our previous observation of a quasi-reciprocal expression pattern of *Brca1* and *Nbr1* in mouse spermatogenesis, we estimated here the relative mRNA expression of *BRCA1*, *NBR1* (next-to-*BRCA1*) and *NBR2* genes in a panel of permanent cell lines and primary cell cultures derived from human breast cancer or normal mammary tissue. The analysis revealed highly significant downregulation of *BRCA1* in 11 out of 12 examined tumour cell lines and primary cell cultures as compared to non-malignant mammary cells. Two isoforms of *NBR1(1A)* and the classical *NBR1(1B)* transcripts were found in cells from malignant mammary tissues, all of them downregulated in respect to normal cells. The expression of *NBR2* differed, being increased in three permanent tumour cell lines and slightly decreased in all primary breast cancer cell cultures. The *in silico* analysis revealed two new putative domains of the predicted *NBR1* protein, suggesting its role in the ubiquitin pathway. The recent identification of the ubiquitin protein ligase activity of BRCA1 implies a possible functional connection between both genes.

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Abbreviations: OPR – octicosapeptide repeat, UBA – ubiquitin-associated domain.

BRCA1 is a tumour suppressor gene frequently mutated in the familial cases of breast and ovarian cancer. It is responsible for about 50% of the hereditary cases of early-onset breast cancer and for more than 80% of familial breast and ovarian cancers (Easton et al., 1993). *BRCA1* encodes a large nuclear, cell-cycle regulated phosphoprotein of 1863 amino acids in size (Miki et al., 1994; Scully et al., 1996; Ruffner and Verma, 1997). Though more than 200 different germline *BRCA1* mutations were identified in the affected families (Rahman and Stratton, 1998), the precise function of the gene in breast cancer initiation and formation is still unclear. However, it appears that the non-mutated form of *BRCA1* is essential for several fundamental processes, namely transcription regulation (Chapman and Verma, 1996), cellular response to DNA damage as a part of DNA-repair complexes (Zhong et al., 1999; Wang et al., 2000) and DNA-damage signalling (Cortez et al., 1999; Tibbetts et al., 2000). Recently, a new role of the *BRCA1* gene was reported in another basic process, the ubiquitin pathway of selective non-lysosomal proteolysis of cellular proteins. The N-terminal RING finger domain of BRCA1 displays a ubiquitin protein ligase activity *in vivo*, which is inactivated by breast cancer mutations (Hashizume et al., 2001; Ruffner et al., 2001). Moreover, it interacts with the ubiquitin carboxy-terminal hydrolase BAP1 (Jensen et al., 1998). The ability of BRCA1 to repress the oestrogen-receptor transcriptional activity (Fan et al., 1999) implies its function in the oestrogen signalling pathway and thus, possibly, in the pathogenesis of breast and ovarian cancer. Decreased levels of *BRCA1* mRNA and protein product were also observed in the sporadic cases of breast cancer, though no mutations of *BRCA1* or its promoter have been found (Futreal et al., 1994; Thompson et al., 1995; Sourvinos and Spandidos, 1998; Catteau et al., 1999b; Wilson et al., 1999). Since the methylation of *BRCA1* promoter was observed only in 11-13% of the sporadic breast cancer cases (Dobrovic and Simpfendorfer, 1997; Magdinier et al., 1998; Mancini et al., 1998; Catteau et al., 1999a; Esteller et al., 2000), other mechanisms of *BRCA1* downregulation have to be sought out.

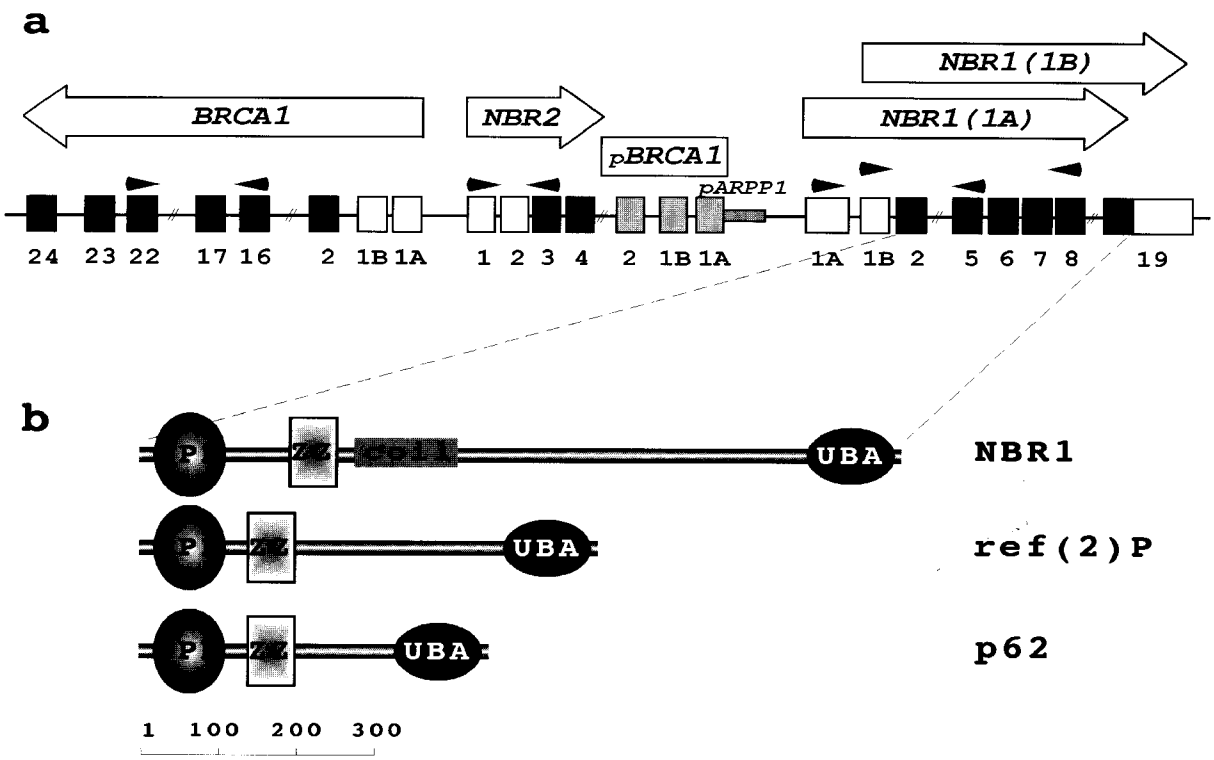

Fig. 1. (a) Schematic representation of the *BRCA1-NBR1* intergenic region on human Chr 17q21. The starts and directions of transcription of *BRCA1*, *NBR1* and *NBR2* genes are shown by arrowed rectangles. Exons, shown as rectangles, are numbered. The ORFs are drawn in black, untranslated sequences are white and the pseudogene is gray. The arrowheads above the exons depict the location of the primers used in this study. The drawing is not to scale. (b) Domain organization of the putative NBR1 protein family. The identification of the novel octicosapeptide repeat (OPR) and ubiquitin-associated domain (UBA) in the NBR1 protein, together with the redefining of the NBR1 zinc finger as a member of the ZZ class of zinc fingers reveal the conserved structure of human and mouse NBR1, *Drosophila* ref(2)P and mouse, rat and human p62 proteins.

The *NBR1* (next to *BRCA1*) gene lies upstream of *BRCA1*, being transcribed in the opposite direction. It was cloned as a candidate gene for the ovarian cancer antigen CA 125 (Campbell et al., 1994), widely used in the monitoring of ovarian cancer. However, the gene lacks the predicted cell-surface characteristics of a secreted protein and its function is not known yet. Although lacking a RING finger domain, the NBR1 protein is held for a member of the RBCC (Ring finger – B-box – Coiled coil domain) protein family, since the identified zinc finger domain was reported to belong to the B-box class of zinc fingers. No mutations of the *NBR1* gene were found in breast cancer (Campbell et al., 1994). The gene is transcribed from two alternative promoters, utilizing two alternative first exons at the 5' end, designated 1A and 1B, respectively. The *NBR1(1B)* transcript is ubiquitously expressed (Campbell et al., 1994), while *NBR1(1A)* expression has been described only in one myeloblast cell line (Nomura et al., 1994). Recently, we have observed *NBR1(1A)/Nbr1(1a)* expression also in normal mouse and human testis, at the postmeiotic, haploid stage of spermatogenesis (Dimitrov et al., 2001).

The structure of the *Brca1-Nbr1/BRCA1-NBR1* interval differs in human and in mouse. We have shown that the mouse *Brca1* and *Nbr1(1a)* are separated by only 289 bp of intergenic sequence (Dimitrov et al., 2001). In humans the genomic region underwent large duplication and rearrangement, resulting in a *pseudoBRCA1* head-to-head with the *NBR1* gene, and a new gene, *NBR2*, partially homologous over its first two exons to *NBR1*, lying 218 bp upstream of *BRCA1* (Fig. 1a) (Barker et al., 1996; Brown et al., 1996; Xu et al., 1997a).

NBR2 is a ubiquitously expressed gene, which encodes a small putative protein of 112 amino acids in size with no homology to other known proteins and with a so far unknown function. No mutations of *NBR2* were found in the examined breast and ovarian cancer cases (Xu et al., 1997a).