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Transcriptional activation of the *ras* oncogenes and implications of *BRCA1* in the cell cycle regulation through *p53* checkpoint

Review article

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Summary

Aberrant expression of *ras* genes has been recognized in several human cancers and is associated with the development of the disease. Thus, revealing the mechanisms that regulate the expression of *ras* genes is critical for understanding their role in the process of tumorigenesis. Transcriptional regulation of the H-*ras* gene, involves nuclear factors recognizing elements in the promoter region of the gene and hormones; so far, a glucocorticoid response element and a p53 element have been identified. Alternative splicing and specific methylation patterns may regulate the expression of *ras* genes as well. Altered expression of *ras* genes has been detected in a variety of human tumours. Differential expression of the *ras* family genes in breast cancer has shown overexpression of all three members of *ras* genes. A significant correlation of overexpression of *ras* genes and stage of the disease was also observed suggesting that aberrant expression of the *ras* genes may be an initial event in breast cancer oncogenesis. Overexpression of Ras p21 protein has been detected in human endometrial and ovarian tumours, due to elevated p53 protein binding on the p53 element of the c-H-*ras* gene, suggesting that p53 protein participates in the development of human gynecological neoplasias through aberrant transcriptional regulation of the H-ras proto-oncogene. Investigation of the *BRCA1* expression levels in relevance with the expression levels of *p53*, *mdm-2* and $p21^{WAF1/CIP1}$ genes, implicated in cell cycle progression, revealed combined alterations of these genes in sporadic breast cancer specimens, indicating that loss of function of *BRCA1* may arrest the cell cycle through *p53* checkpoint.

I. Introduction

The *ras* family genes are among the most well studied and frequently detected genes participating in oncogenesis of human tumors. Three *ras* proto-oncogenes have been so far identified in the mammalian genome: H-*ras* 1, K-*ras* 2 and N-*ras* (Barbacid, 1987). They all encode similar GTP-binding proteins of the same molecular weight (21kDa), termed p21 proteins. These cellular components are associated with the inner face of the plasma membrane, playing, thus, a major role in the transduction of exogenous signals that are essential for the regulation of vital cell functions (Lowy and Willumsen, 1991). The interchange of the p21 proteins between "on" (GTP-bound) and "off" (GDP-bound) position allows them to operate as switches in the cytoplasmic relay of external growth and differentiation signals (Hwang and Cohen, 1997). Interaction of p21 with the Raf oncoprotein results in activation of a cascade of serine/threonine kinases. The intensity and duration of this event strongly contributes to the regulation of cell differentiation and division (Avruch *et al.*, 1994, Marschall, 1995).

II. Activation of the *ras* family genes

ras proto-oncogenes are normally expressed in most human tissues. Mechanisms of activation of these genes are frequently observed in human tumors and mainly include point mutations as well as overexpression of wild-type p21. Point mutations, occurring mainly at codons 12, 13 and 61 of the three *ras* genes, lead to the transformation of the proto-oncogene to an activated oncogene. The mutant p21 loses its ability to become inactivated and, therefore, stimulates cell growth and differentiation constitutively (Kiaris and Spandidos 1995). A plausible explanation for the tendency of *ras* mutations to affect selectively distinct genetic sites is that the cells bearing codons 12, 13 and 61 mutations have a proliferative advantage (Barbacid, 1987). These genetic alterations, consequently, are selected within the cell population as compared to other mutations in different sites of the *ras* genes.

Aberrant expression of the *ras* genes has been recognized in several human cancers and is associated with the development of the disease (Zachos and Spandidos 1997). It is the result of alterations in transcriptional regulation of the genes which quantitatively contribute to the malignant phenotype. *In vitro* experiments have shown that overproduction of even the normal Ras protein is sufficient to give a transforming potential to cultured cells (Spandidos and Wilkie, 1984).

The mechanisms of regulation of *ras* oncogene expression have been widely studied in H-*ras* proto-oncogene. These comprise regulatory elements in the promoter region, regulation by of H-*ras* expression by intronic sequences or by sequences in the 3' end of the gene, as well as interaction of H-*ras* with the p53 tumor-suppressor protein or with steroid hormone receptors. Furthermore, DNA methylation and alternative splicing have been demonstrated to affect H-*ras* gene expression. The proposed models for the regulation of the expression of these genes have been recently reviewed (Zachos and Spandidos 1998).

III. Transcriptional regulation of the H-*ras* gene by the p53 tumor-suppressor protein and by steroid hormone receptors

It has been shown that the H-*ras* proto-oncogene contains in the first intron a p53 binding element that acts as a transcriptional enhancer *in vitro* in the presence of a wild-type p53 protein (Spandidos *et al.*, 1995). Elevated binding of overexpressed wild-type p53 protein has been detected in more than 40% of endometrial and ovarian tumors, compared to the respective normal tissue (Zachos and Spandidos 1998b). Furthermore, overexpression of the *ras* p21 protein in tumor *versus* the adjacent normal tissue correlated with increased levels of intranuclear wild-type p53 and with elevated p53 binding to the H-*ras* element. These findings provide evidence for implication of the H-*ras* gene in gynaecological cancer through aberrant regulation of the p53 protein.

On the other hand, mutated p53 protein was unable to bind to the H-*ras* element. In some of these cases overexpression of the *ras* p21 protein was detected, as well as in certain tumors showing similar levels of p53 binding to the H-*ras*, compared to normal tissue. It has been suggested that regulation of H-*ras* expression in such cases is effected by alternative mechanisms. Elevated steroid receptor binding to corresponding elements of the H-*ras* gene has been observed in more than 90% of gynaecological tumor-normal pairs tested (Zachos *et al.*, 1996). Correlation between *ras* p21 overexpression and estrogen receptor levels in ovarian tumors was also demonstrated (Scambia *et al.*, 1993), moreover, estrogens participate in regulation of the p53 levels (Hurd *et al.*, 1997). Thus, it has been suggested that regulation of H-*ras* expression by steroid hormone receptors and modulation of the H-*ras* gene transcription levels by p53 are inter-related factors in the activation of the gene in human hormone-dependent tumors.

IV. Transcriptional activation of *ras* genes in human breast cancer

Breast cancer is the most common type of cancer in women. It has been suggested that the participation of *ras* genes in breast carcinogenesis consists mainly of expressional activation, since *ras* mutations have been reported infrequently in breast cancer (Rochlitz *et al.*, 1989). Elevated levels of the p21 proteins - encoded by *ras* genes - compared to the respective normal tissues have been detected by immunohistochemical methods in 65-71% of cases (Rochlitz *et al.*, 1989; Spandidos *et al.*, 1992). Recently we examined 27 human sporadic breast cancer specimens analysing the expression levels of tumor *ras* mRNA, compared to respective adjacent normal tissue, using the reverse transcription-polymerase chain reaction (RT-PCR) technique (our unpublished results). Eighteen of the 27 (67%) tumors examined showed transcriptional activation of at least one of the *ras* family genes. N-*ras* exhibited overexpression in 10 (37%), K-*ras* in 9 (33%) and H-*ras* in 10 (37%) of the 27 tumor samples examined. Nine cases (33%) did not present overexpression of any member of the *ras* family genes. Moreover, six (22%) did not show mutational activation of *ras* genes. On the other hand, three samples (11%) exhibited transcriptional activation of all the three *ras* genes, while ten tumors (37%) overexpressed only one, and five (18.5%) overexpressed two of the *ras* genes.

Our experimental data confirm the high incidence of *ras* overexpression reported previously for this type of malignancy (Miyakis et al, submitted). Furthermore, it is shown that overproduction of $p21^{ras}$ is not due to the activation of only one member of the *ras* family, but all three *ras* genes are activated, in various combinations. This ascertainment is enhanced by the lack of correlation between the expression levels of any particular *ras* gene and the clinicopathological parameters of the patients.

Our finding, that *ras* mRNA overexpression is associated with tumors at an earlier stage, is in agreement with observations from related studies on other types of cancer (Kiaris *et al.,* 1995, Vageli *et al.,* 1996). Therefore, aberrant expression of *ras* genes may be an initial event in the breast cancer oncogenetic process. Despite the fact that such alterations seem to play an important role in the primary stages of the disease, *ras* expression is not necessarily required for the maintenance of the transformed phenotype, since mRNA levels tend to decrease in tumors of a more advanced stage. This is further supported by the rather heterogeneous staining of *ras* p21, encountered previously in metastatic breast cancer tissue (Fromowitz *et al.,* 1987).

Clinical data available were insufficient for accurate correlation of *ras* expression with the patients' outcome. Immunohistochemically detected *ras* overexpression has not been found to be significantly associated with time to progression and overall survival (Archer *et al.,* 1995). Nevertheless it has been postulated that oncogene co-expression may serve as a prognostic correlate for recurrence and survival (Bland *et al.,* 1995, Jiang *et al.,* 1997).

V. Transcriptional aberrations of *ras* genes in other types of human cancer.

Apart from its involvement in the oncogenesis of hormone-related human neoplasias overexpression of *ras* genes has been reported in various human tumors. Quantitative molecular biology methods are being applied for the detection of gene expression; these are accomplished at the RNA level using the RT-PCR technique or the RNA spot hybridization analysis, while Western blotting and immunohistochemistry are able to define intracellular levels of the p21 protein. The frequency of the detection of *ras* overexpression varies widely with the stage in the oncogenetic process in which these genetic alterations are believed to be involved (Zachos and Spandidos 1997).

High incidence of augmented *ras* gene expression has been recorded for head and neck carcinomas, as well as for lung and endometrial tumors; *ras* overexpression appears in a later oncogenetic stage in lung and endometrial tumors. On the other hand, *ras* overexpression is considered as a favourable marker for neuroblastomas (Tanaka *et al.,* 1991, Spandidos *et al.,* 1992). Increased levels of *ras* gene expression have been detected in precursors of gastrointestinal neoplasias (Barret's mucosa, colorectal polyps) probably contributing to the malignant transformation of these lesions (Abdelatif *et al.,* 1991, Spandidos *et al.,* 1994). The exhibition of higher Ras p21 protein levels in 30% of a series of thyroid adenomas, compared to normal tissue (Papadimitriou *et al.,* 1988) implicates that elevated *ras* expression may be involved in the conversion of these lesions to carcinomas.

The three *ras* genes exhibit a high incidence of altered expression in numerous human tumors. In addition, a variety of correlations between these genetic changes and major clinicopathological parameters of most types of malignancies have been reported. These features could become useful tool in prognosis and -sometimes- in early diagnosis of human cancer. Understanding the regulatory mechanisms of transcription in these genes, creates new perspectives in the future development of effective molecular strategies for therapy.

VI. Loss of function of BRCA1 may activate the *p53* checkpoint

Extensive studies have revealed underlying mechanisms of *p53* growth suppression and cell cycle regulation. The acidic domain in the amino-terminal region of p53 has transactivation activity (Farmer *et al.,* 1992, Fields *et al.,* 1990). In addition, p53 binds preferentially to specific DNA sequences (El-Deiry *et al.,* 1992, Funk *et al.,* 1992). *p53* may also exert growth suppression by binding to the MDM-2 protein. The interaction between MDM-2 and p53 may modulate the activity of p53 (Momand *et al.,* 1992). p53 suppresses growth by transcriptional activation of $p21^{WAF1/CIP1}$ which inhibits Cdk2, a cell division cyclin-dependent kinase (El-Deiry *et al.,* 1993). Inhibition of Cdk2 stops cell division and inhibits DNA synthesis. These data demonstrate the link between *p53* and the cell cycle and suggest a possible pathway which may be altered during carcinogenesis (Harper *et al.,* 1993).

Several known tumor suppressor genes interact with or negatively regulate the cell cycle machinery (Sherr *et al.,* 1995); *BRCA1* may play an important role in this process. Several properties of BRCA1 and p53 suggest that these two proteins may functionally interact. Both p53 and BRCA1 are tumor suppressor genes that have been implicated in DNA damage response and repair pathways (Levine 1997, Scully *et al.,* 1997, Brugarolas *et al.,* 1997). Both p53 and BRCA1 are physically altered by the cellular response to DNA damage: p53 by stabilization and BRCA1 by hyperphosphorylation (Scully *et al.,* 1997, Kastan *et al.,* 1991). Both p53 and BRCA1 can activate

p21$^{WAF1/CIP1}$ as a common target gene (El-Deiry *et al.*, 1993, Somasundaram *et al.*, 1997).

Investigation of the *BRCA1* expression levels in relation to the expression levels of p53, mdm-2 and *p21*$^{WAF1/CIP1}$ genes implicated in the cell cycle progression, revealed combined alterations of these genes in sporadic breast cancer specimens (Sourvinos and Spandidos, 1998). Specimens expressing *BRCA1* up to 2.7-fold lower than normal tissues, overexpressed *p21* and *mdm-2* at the same time, whereas specimens expressing more than 2.7-fold reduced *BRCA1* mRNA levels expressed *p21* at high levels and *mdm-2* was unchanged. These results indicate that certain levels of *BRCA1*, even reduced levels of *BRCA1*, are sufficient to upregulate *p21*, when p53 activity is inhibited by its negative regulator, the *mdm-2*. *p53* expression levels were unaffected, although expression of *mdm-2*, a gene coding for a negative regulator of p53 activity, was elevated in some cases. The latter indicates a critical role for *p53*, not at the expression level but in the activity of the gene. Furthermore, specimens exhibiting more than 2.7-fold reduced *BRCA1* levels overexpressed *p21* while *mdm-2* expression was normal, suggesting that *p21* transcriptional activation is due to p53 activity in cases with dramatically decreased *BRCA1* expression.

These findings, obtained in human sporadic breast tumours, support the model proposed here which has been derived from experiments in mouse embryos during embryogenesis; according to this model BRCA1, BRCA2 and Rad51 act as a complex to repair damaged DNA (**Fig. 1**). Abrogation of BRCA1 function, either through mutation or via decreased expression could lead to the accumulation of DNA damage and the subsequent activation of a checkpoint mechanism, resulting in *p53* activation and the upregulation of the p53-responsive gene, *p21*. Increased p21 levels inhibit cyclin-dependent kinases, resulting in cell cycle arrest. In case of overexpression of the MDM-2, the negative regulator of *p53*, *p21* can be transcriptionally activated, directly, by BRCA1, when it is present in sufficient amounts.

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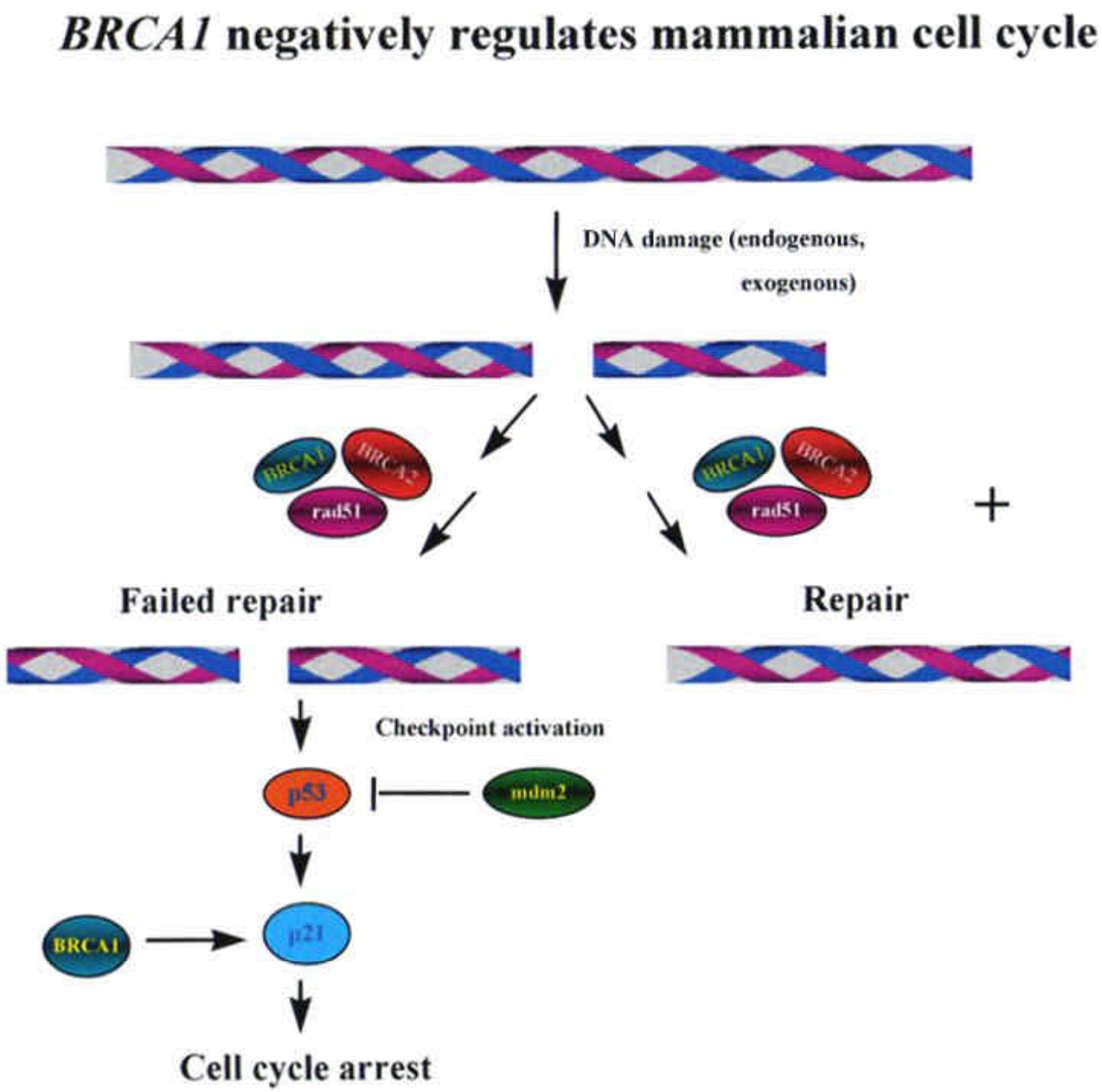

Figure 1. Activation of the p53 checkpoint in response to loss of function of BRCA1.

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