**p21**<sub>WAF1</sub> immunohistochemical expression in breast carcinoma: correlations with clinicopathological data, oestrogen receptor status, MIB1 expression, p53 gene and protein alterations and relapse-free survival

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**Summary** p21 protein (p21) inhibitor of cyclin-dependent kinases is a critical downstream effector in the p53-specific pathway of growth control. p21 can also be induced by p53-independent pathways in relation to terminal differentiation. We investigated p21 immunoreactivity in normal breast and in 91 breast carcinomas [three in situ ductal carcinomas (DCIS) with microinfiltration and 88 infiltrating carcinomas, 17 of which with an associated DCIS; 57 node negative and 34 node positive] with long-term follow-up (median = 58 months). Seven additional breast carcinomas with known p53 gene mutations were investigated. In normal breast p21 expression was seen in the nuclei of rare luminal cells of acinar structures, and in occasional myoepithelial cells. Poorly differentiated DCIS showed high p21 expression, whereas well-differentiated DCIS tumours showed few p21-reactive cells. p21 was seen in 82 (90%) infiltrating tumours; staining was heterogeneous; the percentage of reactive nuclei ranged from 1% to 35%. High p21 expression (more than 10% of reactive cells) was seen in 24 (26%) cases, and was associated with high tumour grade (P = 0.032); no associations were seen with tumour size, metastases, oestrogen receptor status, MIB1 expression and p53 expression. p21 expression in cases with p53 gene mutations was low in six cases and high in one. High p21 expression was associated with short relapse-free survival (P = 0.003).

**Keywords:** p21/WAF1/CIP1; inhibitor of cyclin-dependent kinase; p53

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p21 protein (p21), an inhibitor of cyclin-dependent kinases, is the product of the WAF1 gene (El-Deiry et al., 1993), also known as CIP1 (Harper et al., 1993), SD11 (Noda et al., 1994). p21 is a critical downstream effector in the p53-specific pathway of growth control in mammalian cells. p53 expression in response to DNA-damaging agents promotes the transcription of p21, which causes growth arrest through inhibition of cyclin-dependent kinases (CDKs), which are required for G1 to S transition (El-Deiry et al., 1994; Xiong et al., 1993). p21 can also be induced by p53-independent pathways (Michieli et al., 1994; Johnson et al., 1994; Sheikh et al., 1994), and its expression seems related to induction of differentiation in several cell lines (Jiang et al., 1994; Steinman et al., 1994; Halevy et al., 1995; Zhang et al., 1995). p21 is also expressed in terminally differentiated cells of embryonic and adult mouse tissues (Parker et al., 1995) and in some human tissues (El-Deiry et al., 1995; Marchetti et al., 1996; Doglioni et al., 1996). It has therefore been suggested that p21 may not only be responsible for the p53-mediated growth arrest following DNA damage, but it may also play an important role in maintenance of growth arrest in terminally differentiated cells (Johnson et al., 1994; Halevy et al., 1996). Heterogeneous p21 expression has been observed in various human epithelial neoplasms (Marchetti et al., 1995; Doglioni et al., 1996). In human lung non-small-cell carcinomas, p21 expression at both immunohistochemical and mRNA levels is related to tumoral differentiation and is independent from p53 gene and protein alterations (Marchetti et al., 1996). Conversely, in colorectal cancers, p21 immunohistochemical expression is not related to tumour grade and is inversely related to p53 protein overexpression (Doglioni et al., 1996). These data suggest that p21 expression in human neoplasms may be differentially regulated in a tissue-specific way.

In the present paper we investigated the expression of p21 at the immunohistochemical level in a series of 91 consecutive breast carcinomas. The aim was to evaluate p21 expression in relation to clinicopathological characteristics of the tumours, oestrogen receptor (ER) status, expression of p53 protein and of K67 proliferation related antigen, and relapse-free survival. An additional group of seven breast carcinomas with known p53 gene mutation was also evaluated to further investigate the relations of p21 expression and p53 alterations.

**Material and methods**

**Patients**

A total of 91 consecutive cases of breast carcinomas were investigated; patients had undergone surgery at the S. Chiara Hospital of Trento, Italy (69 cases), or at the John Radcliffe Hospital of Oxford, UK (22 cases), from January 1988 to December 1991. Eligibility criteria were: histological diagnosis of breast carcinoma, level one or complete axillary lymph node dissection, no distant metastases, unilateral tumour. Fifty-seven cases were node negative and 34 cases were node positive (N1 or N2). The median follow-up duration of the patients was 58 months (range 9–128). Node-negative patients did not receive adjuvant therapy, whereas node-positive patients were treated with systemic chemotherapy or hormone therapy and/or radiotherapy.

**Tumour samples**

Surgical samples were collected shortly after surgical removal, and routinely fixed in buffered formalin for 24–48 h at room temperature. Tissue specimens were routinely processed; cases
were classified according to Azzopardi (1979) as follows: 74 infiltrating ductal carcinomas; two infiltrating lobular carcinomas; five infiltrating tubular carcinomas; six mucinous carcinomas, one cribriform infiltrating carcinoma; and three in situ ductal carcinomas (DCIS) with microinfiltration. Invasive carcinomas were graded according to the modified Bloom's grading system according to Elston and Ellis (1991). In 25 cases there was abundant normal breast tissue surrounding the neoplasms. In 17 cases the infiltrating tumour was associated with a DCIS. The 20 cases of DCIS (three DCIS with microinfiltration and 17 associated with an overwhelming invasive component) were classified according to Holland et al. (1994): four cases were well-differentiated DCIS (including one solid, one cribriform and two micropapillary), 11 were intermediate-differentiated DCIS (including five solid, three cribriform, two micropapillary, one clinging) and five cases were poorly-differentiated DCIS (including three comedo and two solid).

Seven additional breast carcinomas (four infiltrating ductal carcinomas, two medullary carcinomas and one infiltrating lobular carcinoma) with known p53 gene mutations were investigated for p21 expression. These cases were selected from a series of 148 previously published cases that had been analysed for p53 mutations using the polymerase chain reaction/single-strand conformation polymorphism (PCR–SSCP) technique and gene sequencing (Marchetti et al., 1993). These seven cases have also been proved to have no p21 gene alterations (Marchetti et al., 1995).

**Immunohistochemistry**

p21 immunoreactivity was evaluated on paraffin sections of primary tumours using the EA10 monoclonal antibody (Oncogene Science, Cambridge, MA, USA), as described previously (Marchetti et al., 1996; Doglioni et al., 1996). Briefly, 4 µm paraffin sections were treated with the microwave antigen retrieval system, incubated for 1 h at room temperature with the primary antibody (1:100 dilution) and processed with the StreptABC technique, using the Duett Kit (Dako, Glostrup, Denmark). Positive controls were sections of lung tumours known to express p21 at the mRNA and protein levels (Marchetti et al., 1995). ER status was evaluated at the immunohistochemical level using the ER1D5 antibody, as described previously (Mauri et al., 1994; Veronese et al., 1995). p53 protein immunoreactivity was assessed with the D07 monoclonal antibody (Novocastra Laboratories, Newcastle upon Tyne, UK) as described previously (Dei Tos et al., 1993). Positive controls for p53 immunostaining were sections of breast carcinomas known to overexpress p53 and sections of atypical fibroxanthoma with known p53 gene mutation and protein accumulation (Dei Tos et al., 1994). Twenty-one cases were immunostained with the MIBI/Ki67 proliferation antibodies related antigen, as described previously (Barbareschi et al., 1994). Negative controls were obtained by omitting primary antibodies.

Cells were considered positive for p21, ER and p53 only when distinct nuclear staining was identified. The percentage of immunoreactive nuclei was evaluated by counting the whole section at medium and high magnification, and by counting at least 500 cells in the most densely stained tumour areas.

Selected cases were processed with a double immunohistochemical technique to stain p21 and p53 or p21 and MIB1, using a StrepABC and an alkaline phosphatase anti-alkaline phosphatase (APAAP) method, as described previously (Doglioni et al., 1996). Briefly, the sections were first immunostained with the first primary antibody followed by a StreptABC technique with 3,3'-diaminobenzidine (DAB, brown reaction product) or aminoethyl-carbazole (AEC-red reaction product) development; subsequently the sections were treated in a microwave oven to block antibody cross-reactivity (Lan et al., 1995) and immunostained with the second primary antibody, using either a StreptABC or an APAAP technique with nitroblue tetrazolium and 5-bromo-4-chloro-3-indol phosphate (NBT/BCIP, blue reaction product) or fast blue cytochemical staining. Negative controls were obtained by omitting primary MAb.

**Statistical analysis**

Statistical analysis was performed using the SAS system (PROC FREQ, PROC LIFETEST and PROC PHREG), run on an IBM-compatible personal computer. The association between the variables was assessed using the chi-square and

Figure 1 Isolated p21-reactive cells in normal breast epithelial cells (a) (arrows) and in cells showing apocrine metaplastic changes (b). p21 immunostaining using the StreptABC technique with DAB development and light haematoxylin counterstain (original magnification (a) × 250 (b) × 400).
Results

p21 immunohistochemistry

p21 immunoreactivity was always nuclear, with only rare faint cytoplasmic staining. In normal breast tissue p21 immunostaining was limited to rare luminal cells of ducts and acinar structures, and to occasional myoepithelial cells (Figure 1a). The percentage of p21-reactive normal cells was usually low (below 1% of the cells), but occasional acinar structures showed a more pronounced p21 immunoreactivity pattern. Staining intensity of p21-reactive normal cells was usually low. Occasional p21-reactive cells were seen in areas of adenosis and in rare apocrine cells lining cystic spaces (Figure 1b). Foamy cells within ectatic ducts were occasionally p21 reactive.

DCIS showed heterogeneous p21 immunoreactivity. The percentage of p21-reactive cells ranged from 0% to 38%, and the median percentage value was 3% (Figure 2). Well-differentiated DCIS showed a low percentage of p21-reacting cells, the mean percentage of p21-reacting cells being 0.75% (range 0–2%). Conversely, poorly differentiated DCIS showed a high percentage of p21-positive cells, the median percentage value being 23% (range 3–38%). In intermediate-differentiated DCIS, the median value of p21-reactive cells was 6% (range 0–20). Subdividing the cases of DCIS on the basis of the median value of the percentage of p21-reactive cells, a clear difference was seen between the three groups of lesions (a formal statistical analysis could not be performed owing to the small number of cases) (Table I).

In the overall series of breast carcinomas, p21-reactive cells were seen in 82 (90%) cases. Staining intensity was variable and heterogeneous (Figure 3). Frequently a mixture of strongly and faintly stained cells was observed, but only clearly positive cells were considered positive. The percentage of p21-reactive nuclei ranged from 0% to 50% of tumour cells; mean ± s.d. and median percentage of p21-reactive cells were 7.6 ± 9.6 and 3.

Twenty-four (26%) cases showing strong p21 immunoreactivity (more than 10% of reactive tumour cells) were considered as expressing high levels of p21. p21 immunoreactivity was seen in all types of infiltrating carcinomas. In some high-grade carcinomas, p21 was strongly expressed in huge atypical nuclei. A statistically significant association trend was seen between high p21 expression and high histological grade (P = 0.032). No association could be seen between high p21 expression and any of the other pathological and biological parameters examined (Table II).

Immunohistochemical staining of the cases with known p53 gene mutation showed that p21 immunohistochemical expression was low in six cases, and high in one case. This case with high p21 expression showed p53 overexpression (immunostaining in more than 90% of tumour cells) and presented p53 gene mutation at codon 273 (exon 8).

In the stromal component of several tumours, p21 immunoreactivity was seen in occasional fibroblast and rare lymphoid cells.

ER nuclear immunoreactivity was seen in 42 (46%) carcinomas. Thirty-four (37%) cases with more than 10% of reactive nuclei were considered as having a positive ER status. p53 immunoreactivity was seen in 41 (45%) cases: staining was always nuclear and the percentage of p53-reactive nuclei ranged from 0% to 95%. Fourteen (15%) cases with more than 15% of p53-reactive nuclei were considered as overexpressing p53.

Cases with high p53 and p21 expression were investigated using double immunostaining: most nuclei were intensely blue or brown (blue or red, depending upon the immunostaining technique) (Figure 4a) but some nuclei showed intermediate colours, suggesting that some cells can accumulate both gene products (Figure 4b). Double immunostaining for p21 and MIB1 showed that the two antigens were mutually exclusive (Figure 5).

Clinical outcome of the patients

Only relapse-free survival (RFS) was evaluated in the present study as the number of deaths due to disease progression did not reach the statistical significance level.
Table I Relations between histological types of DCIS [according to Holland et al. (1994)] and p21 expression

| Histological type     | Low p21 expression\(^a\) (%) | High p21 expression (%) |
|-----------------------|------------------------------|-------------------------|
| Type 1 (well differentiated) | 4 (100%)                    | 0 (0%)                  |
| Type 2 (moderately differentiated) | 4 (36%)                    | 7 (64%)                 |
| Type 3 (poorly differentiated) | 0 (0%)                      | 5 (100%)                |

\(^a\)Case 211 p21 expression was considered low if the percentage of p21-reactive nuclei was less than the median value of 3%.

Table II Relations between p21 expression and biological and clinical variables in breast carcinomas

| Variable          | Low p21 expression\(^a\) (%) | High p21 expression (%) |
|-------------------|------------------------------|-------------------------|
| Histology         |                              |                         |
| Ductal            | 52 (71)                      | 22 (29)                 |
| Other\(^b\)       | 15 (83)                      | 3 (17)                  |
| Tumour size       |                              |                         |
| <20 mm            | 37 (76)                      | 12 (24)                 |
| >20 mm            | 30 (71)                      | 12 (29)                 |
| Nodal status      |                              |                         |
| Positive (N1,N2)  | 23 (68)                      | 11 (32)                 |
| Negative (N0)     | 44 (77)                      | 13 (23)                 |
| Grading\(^d\)     |                              |                         |
| G1                | 15 (100)                     | 0 (0)                   |
| G2                | 26 (76)                      | 8 (24)                  |
| G3                | 25 (66)                      | 13 (34)                 |
| ER status\(^e\)   |                              |                         |
| ER positive       | 28 (82)                      | 6 (18)                  |
| ER negative       | 39 (68)                      | 18 (32)                 |
| p53 expression\(^f\) |                      |                         |
| p53 positive      | 10 (71)                      | 4 (29)                  |
| p53 negative      | 57 (74)                      | 20 (26)                 |
| MIB1 expression\(^g\) |                      |                         |
| Low MIB1          | 10 (63)                      | 6 (37)                  |
| High MIB1         | 11 (69)                      | 5 (31)                  |

\(^a\)Cases are considered to have low p21 expression when p21-labelled nuclei are <10%, and are considered with high expression when more than 10% of the nuclei are p21 reactive. \(^b\)Including lobular, tubular, mucinous, cribriform infiltrating carcinomas and DCIs with micro-infiltration. \(^c\)Not statistically significant. \(^d\)Grading not performed in three DCIS with microinfiltration and in one infiltrating ductal carcinoma whose morphology was not well preserved. \(^e\)ER status was considered positive if at least 10% of tumour cells showed nuclear immunoreactivity. Cases with more than 15% p53-positive tumour cells were considered as overexpressing p53. \(^f\)MIB1 immunostaining data were available for only 32 cases of node-negative breast carcinomas; MIB1 labelling was considered low if the percentage of reacting cells was below the median value of 15%; MIB1 labelling was considered high if the percentage of reacting cells was > to the median value.

![Figure 3](https://via.placeholder.com/150)

Figure 3 Examples of breast carcinomas with different percentages of p21-reactive cells. (a) Grade 1 infiltrating ductal carcinoma with less than 1% of reactive cells. (b) Grade 2 infiltrating ductal carcinoma with 5% of reactive cells. (c) Grade 3 infiltrating ductal carcinoma with strong p21 reactivity in 18% of tumour cells. p21 immunostaining using the StrepABC technique with DAB development and light haematoxylin counterstain (original magnification ×250).

not allow a reliable statistical analysis. Disease relapses were seen in 14 out of 57 node-negative patients and in 13 out of 34 node-positive patients.

At univariate analysis high p21 expression (more than 10% of reactive cells) proved to be statistically related to short RFS (Figure 6, \(P = 0.003\) log-rank test). Besides p21 expression, large tumour size, presence of lymph node metastases, negative oestrogen receptor status and high grading were also significantly predictive for short RFS (Table III).

Multivariate analysis of the above variables has been performed using three different models. In the first one all variables were dichotomised as shown in Table III: using this model the only independent predictors for short RFS were large tumour size and high p21 expression (\(P = 0.0065\), risk ratio 3.072 and \(P = 0.0061\), risk ratio 2.285 respectively). A second model was fitted forcing the variable nodal status to be added to the model, with the aim of considering its potential influence: using this model large tumour size and high p21 expression were the only independent predictors for short RFS (\(P = 0.0061\), risk ratio 3.089 and \(P = 0.0089\), risk ratio 2.757 respectively), whereas nodal status was not far from significance (\(P = 0.0887\), risk ratio 2.002). A third model
Figure 4 Double immunostaining for p21 (brown) and p53 (blue) in a case of infiltrating ductal carcinoma with known p53 gene mutation in exon 8. p21 immunoreactivity was low (5% of labelled nuclei) whereas p53 was diffuse and homogeneous. Some nuclei are golden brown as they express only p21, and others are dark blue as they express only p53; there are however also some other nuclei that show an intermediate brownish-blue colour, suggesting that they express both antigens. p21 immunostaining was done using the StrepABC technique with DAB (brown) development; p53 immunostaining with StrepABC technique and NBT (blue) development (original magnification ×400).

Discussion

The p21 inhibitor of cyclin-dependent kinases is involved in terminal differentiation of several cell systems, and in p53-dependent inhibition of cell cycle progression (El-Deiry et al., 1993; 1994; 1995; Noda et al., 1994; Xiong et al., 1993; Michieli et al., 1994; Parker et al., 1995; Halevy et al., 1995). Here we present evidence that in normal breast epithelium p21 is expressed in rare (<1%) luminal cells of ducts and acinar structures, and in occasional myoepithelial cells, was used, considering the grading as a numerical variable; using this latter model the only independent predictors for short RFS were grading and nodal status (P = 0.0065, risk ratio 2.522 and P = 0.0409, risk ratio 2.420), while p21 was excluded with a P-value of 0.1942. The above different results obtained with different multivariate analysis models, suggest that the effect of p21 may be at least partially dependent on its strong association with grading. However, these data are to be considered as preliminary as the small and heterogeneous number of cases in the present series may bias the survival analysis (Figures 7 and 8).

Table III  Relapse-free survival analysis

| Variable       | P-value (log-rank test) |
|----------------|------------------------|
| Tumour size    | <20 vs >20mm           | 0.003                  |
| Grading        | 1 plus 2 vs 3          | 0.01                   |
| Nodal status   | Positive vs negative   | 0.03                   |
| Histotype      | IDC vs other           | 0.03                   |
| ER             | Positive vs negative   | 0.048                  |
| p53            | Positive vs negative   | 0.13                   |
| p21            | <10 vs >10             | 0.003                  |
whereas most epithelial cells are unreactive. This is at variance with other human epithelial systems, such as the colonic epithelium, where most cells in the upper third of the glandular cryptae and in surface epithelium (i.e. maturing and terminally differentiated cells) are p21 reactive (Doglioni et al., 1996; El-Deiry et al., 1995). It is tempting to hypothesise that these different patterns of p21 expression may reflect different mechanisms regulating cell proliferation, differentiation, quiescence and apoptosis in different epithelial systems (El-Deiry et al., 1995). The differences in p21 reactivity in breast and colonic epithelium may indeed be related to their different physiological properties. Breast epithelial cells have a low proliferation and apoptotic rate, which reach a peak toward the end of the menstrual cycle and are influenced by rhythmic hormonal and/or growth factor changes during the menstrual cycle (Ferguson and Anderson, 1981; Going et al., 1988; Sabourin et al., 1994); conversely, colonic epithelial cells have high turnover, with a continuous high proliferation and cell loss rate (Levine and Haggitt, 1992). Assuming that p21 expression is related to cell differentiation and exit from the cell cycle (Johnson et al., 1994; Halevy et al., 1995; El-Deiry et al., 1995), it might be hypothesised that in the low-turnover breast epithelial system, the low percentage of p21-positive maturing cells parallels the low percentage of proliferating and dying cells; conversely in the high-turnover colonic epithelium, the percentage of p21-positive maturing cells parallels the high percentage of proliferating and dying cells. There are indeed several other differences in the expression and regulation of genes involved in proliferation and apoptosis in breast and colonic epithelium. For example, Bcl-2 gene product, which is known to prevent the apoptotic cascade, is widely expressed in normal breast epithelium, whereas it is confined to only a few cells in the deeper portions of colonic cryptae (Sicinipo et al., 1995; Sabourin et al., 1994; Doglioni et al., 1994; Gasparini et al., 1995).

In DCIS high p21 expression is more frequent in high-grade lesions characterised by the presence of abundant apoptotic bodies and by a typical pattern of central cell death. Cell death in these types of DCIS could be related to ischaemia, due to cell growth in the absence of neoangiogenesis. It is tempting to hypothesise that the ischaemia-dependent growth arrest of DCIS cells could be accomplished by induction of p21. Indeed some of the autocrine/paracrine factors with growth-inhibitory properties, such as transforming growth factor (TGF-β1 (Gorsch et al., 1992; Bursch et al., 1993), may also induce p21 expression as shown in some in vitro epithelial systems (Datto et al., 1995).

Our present data on infiltrating breast carcinoma suggest that p21 altered expression may be of pathogenetic relevance, high p21 expression being associated with tumour progression.

Several mechanisms may be responsible for p21 altered induction and heterogenous expression at the immunohistochemical level. Alterations of the WAF1/CIP1 gene could be one of these mechanisms, but to date no WAF1/CIP1 gene mutations have been reported in breast carcinomas (Marchetti et al., 1995; Shiohara et al., 1994). Alterations in the p21 induction pathway could be an alternative mechanism. p21 may be induced by wild-type p53 (El-Deiry et al., 1994; Xiong et al., 1993): breast cancer cell lines expressing wild-type p53 gene constitutively express 26 to 33-fold higher p21 mRNA levels than cells harbouring the mutant p53 gene (Sheikh et al., 1994). It could be hypothesised that heterogenous p21 expression at the immunohistochemical level may reflect different p53 functional status, high p21 expression being related to normal or increased p53 function, and low p21 expression being related to inactivation of p53 function. This mechanism has indeed been hypothesised to explain p21 expression in colonic carcinomas (Doglioni et al., 1996; El-Deiry et al., 1995). However, in the present series of cases no definite relation was seen between p21 expression and p53 immunohistochemical alterations. Cases with low p21 and p53 expression could be explained on the basis of the above hypothesis. However there were cases with concurrent high p21 and p53 expression: as p53 overexpression is almost always due to p53 gene mutation and possibly p53 function inactivation, it may be hypothesised that p53-independent mechanisms may indeed be responsible for p21 expression in breast carcinomas. These data are in keeping with the findings in lung carcinomas of the non-small-cell type, where p21 expression is independent from p53 alterations and p53 protein expression (Marchetti et al., 1995).

p21 heterogeneous expression could be related to the p53-independent p21 transcription pathway related to terminal differentiation. Expression of p21 (at mRNA and protein levels) has indeed been demonstrated during induction of differentiation of several tumour cell lines (Jiang et al., 1994; Steinman et al., 1994). Moreover, in lung carcinomas high p21 expression, at immunohistochemical and mRNA levels, is related to tumour differentiation, both in terms of global differentiation of the tumours and in terms of immunolocalisation of p21 in foci of more pronounced differentiation within single tumours (Marchetti et al., 1996). However, in the present series of breast carcinomas high p21 expression was not associated with tumour differentiation: on the contrary, there was a trend for less differentiated tumours to express high levels of p21. An hypothesis to explain such inverse association could be related to the fact that histological grade is a function of nuclear atypia, which in some cases could be related to the age of the cells, and in several cell systems p21 expression increases in an age-dependent way (Tahara et al., 1995). Moreover, recent in vitro data suggest that p21 and p53 can be associated with high p21 expression (Sheikh et al., 1995): another alternative hypothesis concerns the possible induction of p21 by TGF-β1, which is known to be associated with disease progression in breast carcinoma (Bursch et al., 1993; Datto et al., 1995).

Our present data on p21 and MIB1 expression are
puzzling: at the single cell level p21 and MBI are mutually exclusive, but no definite relation was seen examining p21 and MBI labelling indexes. p21 expression is indeed related to growth arrest (El-Deiry et al., 1995), and larger studies should further investigate the role of p21 expression and proliferation markers, such as MBI, nuclear proteins expressed in quiescent cells, such as statin (Ansari et al., 1993; Palanca-Wessels et al., 1994) or other inhibitors of cyclin-dependent kinases, such as p27.

Much more has to be learned concerning the role of p21 expression in breast carcinoma. However, regardless of the complexities of the molecular pathways that are responsible for p21 expression in breast carcinomas, our preliminary data suggest that its evaluation could be of possible prognostic value, possibly adding information to that obtained from conventional prognostic parameters.

Moreover, as p21 is an important downstream effector in the p53-specific growth arrest pathway in response to DNA-damaging agents (El-Deiry et al., 1994; Xiong et al., 1993), it is tempting to hypothesise that its heterogeneous expression in tumours may be of relevance concerning the possible therapeutic effects of anti-cancer drugs and radiotherapy that induce DNA damage and/or trigger apoptosis.

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