Evidence for Selective Expression of the p53 Codon 72 Polymorphs: Implications in Cancer Development

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Abstract

Polymorphism at codon 72 of p53 occurs in either the arginine or proline form of p53, whose functional significance in carcinogenesis is controversial. We have investigated if the expression of these p53 polymorphs is selectively regulated, using mRNA from peripheral blood of healthy Asian (Chinese) and the Caucasian (Polish) arg/ pro (arg/pro) heterozygous subjects. Asians were found to preferentially express the pro allele whereas the Caucasians preferentially express the arg allele. On the contrary, about 75% of the heterozygote Chinese breast cancer patients preferentially expressed the arg allele, which rarely contained any somatic mutations. Moreover, histologically normal tissues from Chinese heterozygote breast cancer patients showed selective expression of the arg allele, in contrast to the preferential expression of the pro allele in heterozygote healthy normal breast tissues. Together, the data suggest that the expression of the different p53 polymorphs is selectively regulated in different ethnic populations, and that the arg allele is activated during cancer development in Asians. Thus, the expression status of the p53 polymorphs, rather than the genotypic status, might be a useful indicator for cancer susceptibility. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2245–52)

Introduction

Mutations in the p53 gene are considered to represent the most common genetic alterations in human cancers (1). In addition to gene mutations, several reports have focused on p53 polymorphisms as risk factors for the malignant disease. A polymorphic site at codon 72 in exon 4 encodes either an arginine amino acid (Arg) or a proline (Pro) residue (2). This polymorphism is located in a proline-rich region of the p53 protein that is required for the growth suppression and apoptosis mediated by p53 (3). The two polymorphic variants of wild-type p53 have been shown to have different biochemical properties such as differential binding to components of the transcriptional machinery (4). Moreover, it has also been suggested that the Arg variant is much more susceptible to degradation by the human papillomavirus E6 protein (5), and recent analysis has shown that the Arg variant is more efficient in inducing cell death than the Pro variant in some cell types (6). Although biochemically different, the significance of the p53 codon 72 polymorphism remains controversial in terms of cancer epidemiology. Significant association between the codon 72 polymorphism and risk of cancer has been reported, although the results with regard to most cancer diseases, including breast cancer, remain inconclusive (7-9). Nonetheless, it is possible that the differences between various reports can reflect the populations that were analyzed, as there are inherent differences in the relative prevalence of the polymorphic alleles in various populations (10). A strong correlation is apparent between the p53 codon 72 polymorphism and ethnicity, with the frequency of the arginine (arg) allele being more predominant in populations who are farther away from the equator (10).

Hitherto, no study, to our knowledge, has evaluated the expression status of the different p53 polymorphic alleles, especially in healthy and cancer populations. We reasoned that there might be differences in the expression status of the different p53 codon 72 polymorphic alleles, which could be a determining factor in cancer susceptibility. As such, we have evaluated the status of the expressing allele in healthy Asian (Chinese) and Caucasian (Polish) populations and compared these data to that of the Chinese breast cancer subjects. Our data suggest that there is preferential expression of the different alleles, depending on the population type. In addition, the silent arg allele in healthy Chinese heterozygotes seems to have been reactivated during breast cancer formation, suggesting a positive correlation between arg allele expression and cancer susceptibility. Detailed results are discussed.

Materials and Methods

Materials. Peripheral blood was obtained from a total of 160 Chinese (11) and 105 Polish healthy subjects. Ninety-four Chinese breast cancer samples and histologically matched normal tissues, which have been used in our previous study (12), were obtained from the National Cancer Center tissue repository on written approval from the repository management and ethics committee. DNA and mRNA were prepared according to standard procedures using commercial kits. Polish blood samples were lysed and transported in RNA-Later reagent (Qiagen) before RNA extraction.

Genotyping, Mutational Analysis, and Sequencing. Genomic DNA from peripheral blood samples was analyzed for the genetic variation in codon 72 in exon 4 of the p53 gene by PCR analysis followed by BstU1 digestion, using primers described in Table 1. The p53 arg allele has a unique BstU1 site that is absent in the pro allele, resulting in bands of different sizes (Table 1). mRNA was used for expression analysis, subsequent to cDNA conversion, using p53-specific reverse
transcription-PCR (RT-PCR) primers. PCR products were run on agarose to visualize the expected bands (Table 1). Sequencing reactions were done using Big Dye Terminator version 3 (Applied Biosystems) and ABI 377 DNA sequencer (Applied Biosystems) according to the instruction of the manufacturer, subsequent to RT-PCR reactions using four primers which amplify in an overlapping manner in the p53 gene, as indicated in Table 1. p53 polymorphic and mutational status was analyzed using BLAST 2 Sequence program from National Center for Biotechnology Information website.

The ratio of pro/arg expression was determined based on the electrophenogram data from the sequencing analysis. Equal peak intensity (of C or G nucleotide) from heterozygotes RNA was set as Pro/Arg = 1.

Statistics. Fishers’ exact test was used to test for statistical significance of the differences in the arg and pro allele status, and only those with P < 0.05 were considered significant. All statistical analyses were done using STATA statistical software 7.0 (Stata Corporation, College Station, TX).

Cryostat Sectioning, Staining, and Histologic Analysis. Snap-frozen breast tissue samples (paired normal and tumor, and normal samples from cosmetic surgery cases) were sectioned using the cryostat to obtain sections varying between 8 and 12 µm in thickness. H&E staining was done on these cryostat sections and tumor content/status of tissue was scored by a senior pathologist.

Results

Preferential Expression of the p53 Codon 72 arg or pro Allele in Different Ethnic Populations. Although most epidemiologic studies evaluate the status of the genotypes of polymorphic genes, we reasoned that it is imperative to evaluate the status of the expressing allele—especially when the heterozygote population is large—which might indicate any allele-specific preferential expression. Peripheral blood genomic DNA from 160 Chinese and 105 Polish healthy donors was used to initially determine the genotype by BstU1 restriction digestion, as well as sequencing, to identify pro/arg heterozygotes. The genotypic frequencies were for Chinese: 35.0% arg/arg, 45.0% arg/pro, and 20.0% pro/pro, versus for Polish: 48.6% arg/arg, 45.7% arg/pro, and 5.7% pro/pro, confirming previous reports that the Caucasians tend to consist of more arg/arg homozygotes whereas Asians tend to have a larger proportion of pro/pro homozygotes (10). The genotypic frequencies of the Chinese and the Polish were not significantly different from that predicted by the Hardy-Weinberg equation (data not shown). mRNA was prepared randomly from 25 Chinese and 48 Polish arg/pro heterozygote samples and the status of the p53 expressing allele was determined. Two independent methods were used to determine the degree of the expression of the various alleles: the PCR/BstU1 restriction digestion method and the sequencing method. We first did reconstitution experiments using varying

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**Table 1. Primers used in this study**

<table>
<thead>
<tr>
<th>Primer</th>
<th>PCR product (bp)</th>
<th>Fragment sizes (bp) after BstU1 digestion</th>
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<td></td>
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<td>Primer for genomic DNA</td>
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<tr>
<td>Sense: 5’TGACTGTCGGCATGTT-3’</td>
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<td>700, 500</td>
</tr>
<tr>
<td>Antisense: 5’-AACAGCTTTGAGTGGAAGGA-3’</td>
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<td></td>
</tr>
<tr>
<td>RT-PCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sense: 5’-ATGGAGAGGGCGAGTCAGATCCTTA-3’</td>
<td>880</td>
<td>880</td>
</tr>
<tr>
<td>Antisense: 5’-TGCTGACGTAGGCCCTTGTCTTGTA-3’</td>
<td>1,200</td>
<td>700, 500</td>
</tr>
<tr>
<td>Primers to sequence p53 gene</td>
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<td></td>
</tr>
<tr>
<td>p53(1) forward: 5’-TGACTGTCGGCATGTT-3’</td>
<td></td>
<td></td>
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<tr>
<td>p53(800) forward: 5’-AACAGCTTTGAGTGGAAGGA-3’</td>
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**Figure 1.** Preferential expression of the p53 pro or arg allele in Chinese and Polish germ line arg/pro heterozygotes. A. Sensitivity of the mRNA digestion method. Reconstitution experiments were carried out with the indicated amounts of mRNA from both the pro and arg samples, mixed as indicated, and samples were subjected to RT-PCR reaction and then to BstU1 restriction enzyme digestion. *pro band of 500 bp; the arg allele gives rise to the indicated doublets of 220 and 280 bp (@). The internal control band spanning exons 5 to 11 gives rise to a 750 bp band (†), which serves as a loading control. B. Determination of sensitivity of the sequencing method. The above mixed samples were also subjected to sequence analysis for determination of the codon 72 of p53. Arrows, presence of the different peaks. Both peaks are detectable even at the lowest ratio, indicating the sensitivity of this method in revealing the presence of both alleles expressed at various allelic ratios. C. Restriction digest analysis of mRNA from pro/arg heterozygote samples. mRNA from representative Chinese and Polish samples was subjected to RT-PCR reaction and then to BstU1 restriction digestion, as described in A. D. Sequence analysis of the expressing allele. Left, electrophrograms of sequence analysis using genomic DNA (gDNA) from all three genotypes. The electrophenogram shows the presence of the C nucleotide as blue peaks and the G nucleotide as black peaks. The presence of both the C (pro allele) and G (arg allele) nucleotides in the genomic DNA of heterozygotes is indicated with arrows and underscores. Right, representative sequences from mRNA of Chinese and Polish heterozygote samples. Arrows, presence/absence of a nucleotide in the heterozygote mRNA samples. E. Ratio of pro/arg expression. The ratio of pro/arg allele expression was determined using sequencing data from 25 Chinese and 48 Polish heterozygote samples. Equal expressions of C and G peaks are represented as 1. Unequal expressions are classified as >1 (more pro than arg) and <1 (more arg than pro). The percentage of people preferentially expressing the pro allele is significantly higher than the arg expressers in the Chinese healthy heterozygote population whereas the number of people preferentially expressing arg allele was significantly higher in the Polish population (P = 0.006, exact test). F. Comparison of genotypic status versus expression status. Left, proportion of the different genotypes in the two populations. Right, status of the expressing allele (pro alone, arg alone, or both pro and arg). The percentage of people preferentially expressing the pro allele is significantly higher than the arg expressers in the Chinese healthy normal population, and vice versa for the Polish population (P < 0.001, exact test). Sample size: Chinese, 160; Polish, 105. G. Total p53 mRNA levels. Total p53 mRNA levels were determined in several samples of all the three genotypes by RT-PCR. Bottom, gapdh levels, used to control for loading. H, arg/pro heterozygotes; P, pro/pro; and R, arg/arg. Heterozygotes are underlined.
ratios of the p53 pro mRNA and p53 arg mRNA as templates in restriction enzyme digestion and sequencing analysis to rule out the lack of sensitivity that might lead to the preferential detection of either the pro or arg alleles (Fig. 1A and B). We found that the presence of even 10% to 20% of either pro or arg in the total template mixture could be detected by restriction digestion analysis (Fig. 1A) and by sequencing (Fig. 1B, see arrows pointing to traces of the peaks), indicating that unequal and varying expression of both alleles can be detected by the methods employed in this study. Using these methods, all the Chinese heterozygote samples were found to preferentially express the pro allele, or expressed both the pro and arg alleles (representative samples shown in Fig. 1C and D). However, there was a distinct absence of the preferential expression of the arg allele in the healthy Chinese heterozygotes (Fig. 1C and D). In some of the heterozygote samples that preferentially expressed the pro allele, even minute amounts of the G nucleotide encoding for an arg allele were not detectable.
indicating selective expression of the pro allele (see Fig. 1D, see arrows). Hence, all the healthy Chinese germ line heterozygotes either preferentially expressed the pro allele or expressed both the pro and arg allele, but not the arg allele alone. By contrast, most of the Polish heterozygotes preferentially expressed the arg allele or expressed both the pro and arg alleles, but never the pro allele alone (Fig. 1C and D). The selective expression of the different alleles is distinctly apparent when the ratios of the pro to the arg alleles were plotted, as determined by the sequencing method. Eighty percent of the Chinese pro/arg heterozygotes primarily expressed the pro allele (pro/arg: >1) whereas about 55.3% of the Polish expressed the arg allele (pro/arg: <1; \( P = 0.006 \), exact test; Fig. 1E). The sequencing method is specific in determining the amount of the C or G nucleotide in the RNA samples, as we have always noticed equal expression of both the C and G peaks using genomic DNA samples, which contain equal amounts of both alleles (Fig. 1D). Thus, the selective expression of the different alleles in the heterozygotes resulted in a significant increase in the number of pro expressers to 56.0% [i.e., from pro/pro and pro/arg genotypes: 20.0% + (80% of 45.0) = 56.0%] in the overall Chinese population and an increase in the numbers of arg expressers to 73.9% [i.e., from arg/arg and pro/arg genotypes: 48.6% + (55.3% of 45.7) = 73.9%] in the Polish (\( P < 0.001 \), exact test), as compared with the conventional genotypic expression pattern (Fig. 1F). However, there were no significant differences in the total p53 mRNA levels, which were variable, among the three genotypes in the Chinese population (Fig. 1G). Thus, there seems to be a bias in the expression of the pro or the arg alleles in the different populations.

**Expression of the p53 Codon 72 arg Allele Is Up-Regulated in Breast Cancer Tissues.** Because there was a preference against the selective expression of the p53 arg allele in the healthy Chinese heterozygote population, we investigated if there was a correlation between susceptibility to cancer and the expression status of p53 polymorphism at codon 72. Ninety-four Chinese breast cancer patient samples and the adjacent, histologically normal epithelial tissues for some samples were screened extensively for mutations in the entire coding region of the p53 gene, as well as for the expression status of the polymorphic variants at codon 72. Allelic expression status analysis revealed that many of the heterozygote samples (15 arg/arg samples analyzed) preferentially expressed the arg allele (Fig. 2A and B), which was in contrast to the data obtained in healthy subjects (Fig. 1C and D). These heterozygote arg expressers did not show any traces of the expression of the pro allele, suggesting that the arg allele is now selectively activated and the pro allele is silenced in the heterozygote cancers. Moreover, none of the heterozygotes from the cancer cohort expressed both the arg and pro alleles (Fig. 2C). Pro/arg ratio analysis in the heterozygote cancer population revealed that about 68% preferentially expressed the arg allele, which was in contrast to the healthy population where preferential arg expression was absent (pro/arg <1; \( P < 0.001 \), exact test; Fig. 2C). We have excluded loss of heterozygosity (of the pro allele) in the heterozygotes as a possibility for the selective expression of the arg allele (Figure 2D, showing representative samples). Furthermore, there was a significant decrease in the number of heterozygotes selectively expressing the pro allele alone in the cancer cohort, a finding that was in contrast to the healthy population (pro/arg >1: normal versus cancer, 80.0% versus 32.0%; \( P < 0.001 \), exact test; Fig. 2C). However, no significant differences in the genotypic frequencies between the normal and breast cancer groups among the arg and pro homozygotes were evident (arg/arg: healthy—35.0% and cancer—43.5%; pro/pro: healthy—20.0% and cancer—12.5%; \( P = 0.096 \), exact test; Fig. 2E, left). The differences in the expression status of the heterozygotes resulted in an increase in the total numbers of arg expressers in the cancer population (i.e., from arg/arg and arg/pro genotypes). Thus, more than half of the tumor samples analyzed, about 73.4% [i.e., (68.0% of 44) + 43.5%], preferentially expressed only the p53 arg allele, compared with 35.0% in healthy subjects (\( P < 0.001 \), exact test; Fig. 2E, right). Concomitantly, the percentages of breast cancer patients expressing both the pro and arg alleles and those preferentially expressing the pro allele were reduced compared with healthy subjects (Fig. 2E, right). The data together indicate that the p53 arg allele is significantly overexpressed in the breast cancer tissues, and hence, suggests that the arg expression is congruent with breast cancer susceptibility.

We also evaluated the mutational status of the p53 gene in the breast cancer samples. Mutations were significantly reduced and detected in only 31% of arg-expressing tumor samples whereas about 55% of the pro-expressing cancer samples had a mutation in the p53 gene (Fig. 2F, \( P = 0.037 \)). Detailed analysis revealed that 63% of all the mutations in the arg-expressing samples were recessive mutations that were predominantly found outside the DNA-binding domain of p53 (Fig. 2G). Of the mutations found outside the DNA-binding domain, about 25% of them were on codon 80 which is in the proline-rich region, resulting in the substitution of a proline to serine residue (Fig. 2G, data not shown). This mutation results in the loss of yet another proline residue in the proline-rich domain, which has been shown to be essential for the induction of apoptosis (3). In contrast to the arg-expressing tumors, 92% of the p53 pro-expressing tumors carried a mutation in the DNA-binding domain, a finding that correlates with the established notion that most mutations in the p53 gene occur in the DNA-binding domain (1). Together, the data show that the codon 72 arg polymorphism of p53 is less targeted for mutations compared with the p53 pro polymorphism in the Chinese population.

**Histologically Normal Tissues from Chinese Breast Cancer Patients Preferentially Express the arg Allele.** We also evaluated the p53 expression status in the corresponding adjacent histologically normal tissues of some of the heterozygote cancers, which were of the luminal type (12). This analysis again revealed that the arg allele was preferentially expressed in the histologically normal tissues (either arg alone or arg and pro) and there was no preferential expression of the pro allele (representative data shown in Fig. 3A and B), indicating selective activation of the arg allele in the histologically normal adjacent tissues. To explore if this was a tumor-associated phenomenon and to exclude the possibility that normal breast tissues from healthy donors (those who undergo cosmetic surgery) also preferentially expressed the arg allele, we analyzed healthy breast tissues from such heterozygote donors. Limited analysis using two healthy arg/pro heterozygote breast tissues revealed that the pro allele was preferentially expressed, although the arg allele was detectable (Fig. 3A and B). However, there was no preferential expression of the arg allele alone (Fig. 3A and B). This was similar to the results obtained with peripheral blood samples (data not shown). Histologic analysis of the histologically normal tissues from tumor patients were indeed phenotypically untransformed and benign, similar to the normal healthy breast tissues and in contrast to the breast tumor tissues (Fig. 3C). Hence, these data suggest that the selective expression of the arg allele in the histologically normal tissues of cancer patients could indicate that the cellular transformation process might have already taken place in these tissues.

**Comparison of p53 Codon 72 Expression Status with Other Markers.** We further investigated if there is a trend between the codon 72 polymorphic expression status and other markers such as the ErbB2 status and estrogen receptor status. Breast cancers were classified, as per our previous study, into ErbB2+ tumors, estrogen receptor-negative tumors, luminal tumors, and normal-like tumors, based on the gene expression profile

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Figure 2. The *arg* allele is preferentially expressed in Chinese breast cancer patients, but is less frequently mutated. **A** and **B**, Cancer tissues from Chinese *arg/pro* patients preferentially express the *arg* allele. Restriction digestion (**A**) and sequencing analysis (**B**) of representative tumor tissues from heterozygote Chinese patients show increased expression of the *arg* allele. **C**, Ratio of *arg/pro* expression in cancer samples. The ratio of *pro)arg* allele expression was determined as described from 25 healthy and 15 cancer heterozygote samples. The percentage of people preferentially expressing the *pro* allele is significantly higher than the preferential *arg* expressers in the healthy normal population whereas the percentage of people preferentially expressing *arg* allele in the cancer population was significantly higher than the normal population (*P* < 0.001, exact test). **D**, Status of *arg/pro* heterozygotes. Genomic DNA from the indicated representative samples from breast tumors was analyzed for loss of heterozygosity by PCR followed by BstU1 restriction enzyme digestion. *, *pro* band of 880 bp; the *arg* allele gives rise to the indicated doublets of 280 and 600 bp (@). The heterozygotes show that both alleles (all three bands) are present. **E**, Comparison of genotypic status versus expression status in healthy and cancer cohorts. **Left**, proportion of the different genotypes in the Chinese healthy and cancer cohorts, which show no significant differences (*P* = 0.096, exact test). **Right**, status of the expressing allele (*pro* alone, *arg* alone, or both *pro* and *arg*) in those samples. The proportion of the cancer patients preferentially expressing the *arg* allele is significantly greater than in the healthy population (*P* < 0.001, exact test). Samples size: normal, 160; cancer, 94. **F**, Comparison of the number of *pro* or *arg*-expressing breast cancer samples with (+) or without (−) a mutation in the *p53* gene. The number of *arg*-expressing patients without a mutation in the *p53* gene is significantly more than those with a mutation (*P* = 0.037, exact test). Samples size: *pro*: 42 (without mutation, 20; with mutation, 22); *arg*: 52 (without mutation, 36; with mutation, 16). **G**, Distribution of *p53* mutations in breast cancers. The top part indicates the *arg* allele and the lower part indicates the *pro* allele in the breast cancer samples, and the frequency of mutations is indicated as length of the bars (not to scale). The frequency of mutation at codon 80 was 25% of the *arg*-expressing tumors.
Limited analysis indicated that the ErbB2+ tumors tended to contain more arg expressers compared with pro expressers (13 arg versus 5 pro; Table 2). However, the numbers were not statistically significant due to the small population size. Comparison of the other cancer types revealed no significant association to the p53 codon 72 polymorphic allele expression status (Table 2). Moreover, there was no significant association between the tumor type and p53 mutation status (Table 2).

Discussion

To our knowledge, this is the first report that describes the expression status of the p53 allele, with respect to the codon 72 polymorphism. The results presented here indicate that there is preferential expression of either the pro or arg alleles, depending on the ethnicity of the populations. There seems to be a selective pressure against the expression of the arg allele.
in the pro/arg Chinese, who live near the equator. By contrast, most of the Caucasian (Polish) heterozygotes seem to preferentially express the arg allele. These data from the heterozygotes, when combined with the homozygotes (both pro/pro or arg/arg) in the respective populations, indicate that the Chinese population is predominantly pro expressers compared with the Polish who are arg expressers. These findings are different from many earlier studies which relied on genotypic evaluation of populations but which have not investigated the expression status of the large heterozygote populations. The data thus suggest that the selective expression of the different p53 polymorphic variants in different ethnic populations may be a consequence of ecological adaptation.

Because the Caucasians are about 2-fold more prone to cancer than the Asians (13) and because there is a distinct absence of the selective expression of the arg allele alone in the healthy Chinese heterozygotes, we have investigated if the expression of the arg allele in the Chinese might correlate with carcinogenesis. Our data show that there is a significant increase in the number of arg expressers in the Chinese breast cancer cohort, indicating that there is a strong correlation between carcinogenesis and the expression of the arg allele in the Chinese population. However, there was no significant increase in the numbers of arg/arg homozygotes between the Chinese healthy and cancer populations, supporting many previous studies that reported the lack of correlation between a particular genotype and cancer predisposition (8). This raises the possibility that the arg allele may not be functionally involved, but its expression may simply relate to tumorigenesis. Thus, further studies are necessary to examine the causal role of the arg allele in carcinogenesis and to evaluate if other genetic modifiers might be involved, especially in arg/arg homozygote subjects, which might influence the susceptibility to cancer.

As most of the healthy Chinese heterozygotes were preferentially expressing the pro allele, it seems that the arg allele is activated (or expression is induced) in cancer tissues and that the pro allele is somewhat down-regulated, probably through an active mechanism that might be regulating expression from the different alleles. In this respect, it should be noted that several other tumor suppressor genes such as neurofibromatosis type 1 (NF1) and NF2 have been shown to be unequally expressed (14, 15). In addition, the p53-related gene, p73, is imprinted and monoallelically expressed (16). A recent report by Magdinier et al. (17) showed that p53 is methylated at exon 4, in which the codon 72 is found, to a high degree in blastocysts. Our limited preliminary analysis of GC-rich regions in the p53 promoter indicates no evidence for methylation (data not shown). However, we cannot exclude the possibility that p53 is imprinted, resulting in silencing of the arg allele in healthy Chinese individuals, through mechanisms that require further investigation. Nevertheless, there seems to be an allelic switch in the expression of the different p53 codon 72 alleles during cancer development. Moreover, analysis of the histologically normal adjacent tissues from heterozygote cancer patients indicates that the arg allele has already been reactivated in these tissues. This is in contrast to normal breast tissues from healthy heterozygote donors, who primarily express the pro allele, similar to the observations with peripheral blood of the Chinese population. It would thus be interesting to analyze the status of the normal tissues from early-stage ductal carcinoma in situ patients in future studies, to evaluate if the expression profiles have been altered early in the cellular transformation process. In any case, the data presented here suggest that p53 expression status might be used as a molecular signature for cellular transformation process that could have already begun in these histologically normal tissues, especially in the large heterozygous population. Further prospective studies are required to analyze if Chinese patients, whose histologically normal tissues preferentially express the arg allele, go on to develop secondary cancers or relapses, even after surgical removal of the primary tumor tissues.

Another intriguing observation evident from this study is that the arg allele is often not mutated in the breast cancers we have analyzed, suggesting that there may not be a need to mutate the arg allele for the cancers to develop. This suggests that the arg allele itself might be a cancer susceptibility p53 allelic variant that serves as a primary lesion, thus contributing to the development of cancer. Hence, there is very little pressure to “knock down” the DNA-binding function of the arg allele. By contrast, there is a strong genetic pressure to selectively mutate the p53 gene at the DNA-binding domain to knock down the DNA-binding function of the pro allele, as compared with the arg allele, to inactivate it. Thus, one would predict that the p53 Arg variant form is biologically weaker than the Pro form in terms of preventing cellular transformation. Although the p53 Arg has been shown to induce apoptosis better than the p53 Pro form, this effect might be cell type specific (6). Hence, the p53 Pro form might be more efficient in other p53-related functions in inhibiting malignancy. Moreover, 50% of all cancers do not contain a mutation in p53 (1), which might be due to expression of the arg form that often does not contain a mutation (this study). This is also true in the case of Caucasian breast cancer cohort, in which about 70% of all arg/arg homozygous cancers were found not to carry a mutation in the p53 gene (18). Together, the data indicate that cellular transformation can occur without the need to mutate arg p53, and suggest that this might be a cancer-prone allele.

In summary, the data presented here show that there is a selection pressure against the expression of the codon 72 p53 arg allele in the healthy Asian germ line arg/pro heterozygotes, and provide insights to the uniqueness of population-based expression of the p53 tumor suppressor gene. It also shows a strong correlation between the expression of the p53 arg allele and susceptibility to breast cancer development. Thus, we propose that the expression status of the p53 polymorphisms, rather than the conventionally analyzed genomic status, be used as one predictive factor for the predisposition to breast cancers.

**Acknowledgments**

We thank Dr. HH Li for providing statistical assistance and Dr. P.H. Tan for histopathologic analysis. We also thank the National Cancer Centre tissue repository for the breast cancer samples.

**References**


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**Table 2. Comparison of p53 codon 72 expression status with breast cancer subtypes**

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*p53 codon 72 RNA status.*


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