Variation in the human TP53 gene affects old age survival and cancer mortality

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Abstract

Longevity may depend on a balance between tumor suppression and tissue renewal mechanisms [Campisi, J., 2003. Cancer and ageing: rival demons? Nat. Rev. Cancer 3 (5), 339–349]. Mice with constitutively activated p53 are almost cancer free but their life span is reduced and accompanied by early tissue atrophy [Tyner et al., 2002. p53 mutant mice that display early ageing-associated phenotypes. Nature 415 (6867), 45–53]. Replacement of arginine (Arg) by proline (Pro) at position 72 of human p53 decreases its apoptotic potential [Dumont et al., 2003. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat. Genet. 33 (3), 357–365] providing a tool to test for a similar trade-off in humans. Using a formal meta-analysis of the published literature we show that carriers of the TP53 codon 72 Pro/Pro genotype have an increased cancer risk compared to Arg/Arg carriers (p < 0.05). Next, in a prospective study of 1226 people aged 85 years and over we show that carriers of the Pro/Pro genotype have a 41% increased survival (p = 0.032) despite a 2.54 fold increased (p = 0.007) proportional mortality from cancer. It is suggested that human p53 protect against cancer but at a cost of longevity.

Keywords: TP53; Codon 72; Old age survival; Cancer; Tissue renewal

1. Introduction

Ageing is thought to occur through the accumulation of molecular and cellular damage, which arises because of evolved limitations in mechanisms for maintenance and repair of somatic cells and tissues [Kirkwood, 1996; Kirkwood and Austad, 2000]. Survival of most organisms in the natural environment is curtailed by accidental mortality rather than intrinsic deterioration. Under those conditions, there would have been no advantage in investing in higher levels of maintenance than were required to keep the body in good condition for as long as it had a reasonable chance to remain alive. Age-related degenerative processes are seen only at ages that, in the case of humans, were not widely experienced until relatively recently. Accumulated damage causes genomic instability and cellular dysfunction, which in organisms with renewable tissues may result in cancer. The threat posed by genome instability is guarded against by, on the one hand, ‘caretaker’ genes which include genes for DNA repair and are considered longevity assurance genes, and, on the other hand, ‘gatekeeper’ genes which include genes for cell death and cell cycle arrest and are considered tumor suppressor genes (Campisi, 2003). Increased activity of a caretaker gene increases...
genome stability and both reduces cancer risk and postpones ageing. There is a well documented positive association between longevity and DNA repair capacity (Burkle, 2000; Kirkwood, 1989; Promislow, 1994). However, the relationship between gatekeeper gene functions and the ageing process is more complex since taking a cell permanently out of cycle or deleting it through apoptosis may diminish cancer risk but at the same time accelerate age-related loss in tissue cellularity.

Recent results from mouse models have highlighted the idea that longevity may involve an optimal balance of the tumor-suppressive actions of gatekeeper genes and their pro-ageing effects. In mammals, p53 is the most important gatekeeper gene. A variety of intracellular stress signals activate p53 to induce either transient cell cycle arrest with stimulation of repair activities, senescence or apoptosis (Vogelstein et al., 2000). The nature and intensity of the stress signals, and the cellular context determine which response occurs. P53 deficient (p53−/− and p53+/−) mice are highly susceptible to cancer (Donehower et al., 1992). Interestingly however, two of the p53+/− mice that, presumably by chance, did not develop tumors had unusually long lifespans (Donehower, 2002). On the other hand, mutant p53 (p53/+m) mice that have constitutively activated p53 show greatly reduced cancer incidence but faster ageing; their lifespan is shortened and accompanied by accelerated age-related reduction in mass and cellularity of various tissues (Tyner et al., 2002).

It was shown previously that the two variants of a common codon 72 polymorphism (Arg and Pro) in the human TP53 gene (Harris et al., 1986) differ, most notably in the better ability of the Arg variant to suppress cellular transformation (Thomas et al., 1999) and induce apoptosis (Bonafe et al., 2004; Dumont et al., 2003). Here, we used this tool to test whether tumor suppression in humans has a cost in longevity, firstly by analyzing carriers of the three different TP53 genotypes for cancer risks using a formal meta-analysis of the published literature, and secondly by comparing old-age survival and cancer-mortality in a prospective population-based study of very old people. Indeed we found evidence that a trade-off similar to that seen in mice appears to occur in humans.

2. Methods

2.1. Systematic literature review and meta analysis

We searched the National Library of Medicine (PubMed), using the terms p53 AND cancer AND (‘codon 72’ OR ‘cod 72’ OR ‘cod72’ OR ‘position 72’ OR ‘arg’ OR ‘pro’ OR ‘proline’ OR ‘arginine’ OR ‘pro72arg’ OR ‘arg72pro’ OR ‘r72p’ OR ‘p72r’ OR ‘polymorphisms’) The search was limited to human. All abstracts were screened for suitability by two independent researchers. The inclusion criteria were: case-control studies written in English with non-related subjects and sufficient data to calculate odds ratio’s, use of non-tumor material for the genotyping of both cases and controls, no deviation from Hardy–Weinberg equilibrium for the genotype distribution of the controls and the same ethnicity for cases and controls. Of the included articles, all references were checked for suitability using the same criteria.

The PubMed search resulted in a total of 756 articles. After screening the abstracts 191 articles were retrieved. Screening of references of these articles yielded another 12 articles that were retrieved. From the total of 203 articles, six were excluded because they were not in English, 77 were excluded because of inappropriate study design, 37 were excluded because tumor material was used to genotype cases, four were excluded because they were a replication of results reported elsewhere, seven were excluded because the genotype distribution of the control group was out of Hardy–Weinberg equilibrium, and seven were excluded because no data were extractable. In total, 65 articles were included in this meta-analysis. Seven studies were pooled to form a total of three study populations, because of common control groups. Data from 61 study-populations were thus available for meta-analysis.

If several studies used the same control group, the overall risk was calculated using that one control group and the pooled cases of those studies. In studies in which the number of Pro/Pro subjects was zero for cases or controls, zero was replaced by 0.5 to enable calculation of odds ratios. The weight of each study was calculated as the inverse of the variance of the risk estimate.

We assessed heterogeneity, i.e. that the variation in outcome of these studies is due to random error around the ‘true’ estimate, using Chi-square (χ²). Because heterogeneity was absent (p > 0.10), fixed effect pooling was used.

To estimate the unbiased effect we performed a meta-regression analysis (Van Houwelingen et al., 2002). We used a weighted regression model with the log odds ratio’s as dependent variable, the standard error as independent variable and the reciprocal variance as weight factor. In this model, the intercept with the y-axis represents the location of the ideal, unbiased study with infinite number of subjects and a standard error of zero.

2.2. Prospective follow-up study and analysis of patterns of mortality and overall survival

The Leiden 85-plus Study is a prospective population-based study that consists of two cohorts of inhabitants of Leiden, The Netherlands (Bootsma-vander Wiel et al., 2002; Slagboom et al., 2000). For cohort ‘87, people aged 85 years and over were enrolled between 1987–1989 and followed for specific causes of death until October 1996. For cohort ‘97, people of exactly 85 years were enrolled between 1997–1999 and followed for specific causes of death until September 2002. In cohort ‘87, 10 years after the start of the study 598 had died (89%). In cohort ‘97, 5 years
after the start of the study 198 had died (36%). TP53 codon 72 genotypes were obtained for 1226 participants of the Leiden 85-plus Study.

The survival analyses have been proceeded in several stages and were all performed according to standard procedures. In the first stage, we plotted Kaplan Meier cumulative survival (y-axis) against age (x-axis) of carriers for the three different TP53 codon 72 genotypes. For cohort '87, the age of entry varied between 85 years and 100 years, while for cohort '97, the age of entry was 85 years for all subjects. We therefore used left censoring to correct for the delayed entry into the risk set according to age. In the second stage of the statistical analyses, we used sex-adjusted, left-censored Cox regression to calculate proportional hazard ratios. The hazard ratio indicates the relative mortality risk of either the TP53 codon 72 Pro/Pro or Pro/Arg carriers versus the Arg/Arg carriers, the latter being the reference group. As survival is inverse of mortality, survival of the index group compared to the reference group is expressed as 1/hazard ratio. Causes of death were adjudicated according to the tenth version of the International Classification of Diseases (ICD-10; WHO, 1994). SPSS and STATA were used for the statistical analyses.

2.3. Genotyping

TP53 codon 72 genotypes were determined with a Taqman Assay by Design (Applied Biosystems). Assay by Design was used as recommended, except for the following modifications. A qPCR core kit was used (Eurogentec) with 1/3 of the recommended amount of assay mix. Post-PCR florescence measurements were performed on an ABI7900 (Applied Biosystems).

2.4. Ethics

The Leiden 85-plus Study was approved by the medical ethical committee of the Leiden University Medical Center and are in accordance with the Helsinki Declaration of 1975, as revised in 1983. Informed consent was obtained from all participants after the nature and possible consequences of the studies were explained.

3. Results

We performed a systematic literature review on the association of the TP53 codon 72 polymorphism with cancer susceptibility yielding data from 61 study-populations (Supplementary Table 1 online) for meta-analysis. The conservative estimate using fixed effect pooling was an increase in cancer risk of a factor of 1.11 (1.02–1.22, \( p < 0.05 \)) for TP53 codon 72 Pro/Pro carriers compared to Arg/Arg carriers. Fig. 1 presents a funnel plot with the log odds ratio plotted on the x-axis against the weight of the study on the y-axis. The peak of the plot (Liu et al., 2001) represents the most accurate single estimate, the OR (95% confidence interval (CI)) being 1.37 (1.01–1.87, \( p < 0.05 \)). The proportion of studies located left of the peak (43/60) is significantly larger than those located to the right (17/60), which is suggestive of publication bias (Fig. 1). The first, preliminary data on the topic may have contributed to biased publication of studies associating the TP53 codon 72 Arg allele with increased cancer risk. These data showed increased tumorigenicity in nude mice of cells transfected with human p53 codon 72 Arg as compared to Pro (Matlashewski et al., 1987) and suggested increased susceptibility of the codon 72 Arg variant to E6 oncoprotein mediated degradation resulting in increased risk to develop human papilloma viruses (HPV)-associated carcinoma (Storey et al., 1998). Adjustment for publication bias by means of meta-regression analysis further increased the pooled cancer risk estimate of TP53 codon 72 Pro/Pro carriers to 1.30 (1.02–1.65, \( p < 0.05 \)). Thus by both Meta-analysis approaches, the data from a systematic literature review showed that the risk to develop cancer was increased for TP53 codon 72 Pro/Pro carriers compared to Arg/Arg carriers.

To test whether variation in the human TP53 gene also affects old age survival, we analyzed patterns of mortality dependent on the codon 72 polymorphism in 1226 subjects aged 85 years and over who were enrolled in the prospective Leiden 85-plus follow-up study. Our data show that survival of carriers of the Pro/Pro genotype was 1.41-fold increased (95% confidence interval: 1.03–1.92) compared to Arg/Arg carriers (\( p = 0.032 \)) (Fig. 2). There was no survival benefit...
for the Arg/Pro genotype, survival being 0.95-fold lower ($p=0.55$). The survival benefit for the Pro/Pro carriers was shown in both cohort '87 (1.31; $n=671; p=0.06$) and cohort '97 (1.39; $n=555; p=0.38$). Limited numbers of Pro/Pro genotypes and the smaller number of deaths in cohort '97 resulted in reduced power to detect statistical significance in the separate cohorts.

As cancer susceptibility and tumor spectra vary greatly with age (DePinho, 2000), we also determined the causes of death dependent on TP53 codon 72 genotypes in these very old people (Table 1). Proportional mortality from cancer was 29% in Pro/Pro subjects compared to 14% in Arg/Arg subjects ($p=0.007$). The increased proportional mortality from cancer for Pro/Pro subjects was not accompanied by a different cancer spectrum (data not shown). We also found a decrease in proportional mortality for Pro/Pro subjects in the category other causes ($p<0.05$). Within this category, 6% of the Pro/Pro subjects compared to 21% of the Arg/Arg

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Arg/Arg</th>
<th>Arg/Pro</th>
<th>Pro/Pro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>187 (43)</td>
<td>117 (37)</td>
<td>21 (47)</td>
</tr>
<tr>
<td>Infectious disease</td>
<td>61 (14)</td>
<td>42 (13)</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Cancer</td>
<td>60 (14)</td>
<td>55 (18)</td>
<td>13 (29)$^a$</td>
</tr>
<tr>
<td>Other causes</td>
<td>128 (29)</td>
<td>101 (32)</td>
<td>7 (16)$^a$</td>
</tr>
</tbody>
</table>

Specific causes of death as obtained in the combined cohorts. Proportional mortality (95% confidence interval) from cancer is a factor of 2.54 increased (1.19-5.37) in Pro/Pro carriers. Death from other causes includes chronic obstructive pulmonary disease, renal failure, dementia, fracture and senility.

$^a$ Different from Arg/Arg, $\chi^2 p<0.05$.

4. Discussion

The key finding of this study is twofold. First, the data from a systematic literature review showed that the risk to develop cancer was increased for TP53 codon 72 Pro/Pro carriers compared to Arg/Arg carriers. Second, our prospective data show that at age of 85 years and over, the beneficial effects of the TP53 codon 72 Pro/Pro genotype on longevity seem to outweigh its harmful effects on mortality from cancer.
Longevity and mortality from cancer are both intrinsically complex and thus of polygenic nature. Our results demonstrate that TP53 codon 72 is one of the genetic determinants of cancer mortality and survival at old age. We speculate that long-term survival of man critically depends on the capability for tissue renewal and repair. With time, as a secondary consequence of senility phenotypes, tissues fail in maintaining homeostasis and repair. With time, as a secondary consequence of apoptosis, tissues fail in maintaining homeostasis and normal cell numbers, resulting in phenotypes such as atrophy of muscles, skin and lymphoid organs, decreased hair growth, and loss of bone density. In humans, such senility phenotypes are typically only observed at advanced age, which makes it likely that the beneficial effects of the codon 72 Pro variant specifically come into play in the last trajectory of life.

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Appendix. Supplementary material


References


