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A quantitative model of cellular senescence influence on cancer and longevity

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Contrary to the paradigm that cancer incidence increases indefinitely with age, significant data now suggest cancer incidence may markedly reduce beyond age 80 years for humans and beyond 800 days for mice, and is not inevitable. We show that increasing cellular senescence with age is a possible cause of this reduction, since senescent cells are removed from the pool of cells that retain proliferative ability necessary for cancer. We further show that animal interventions appearing to alter senescence, p53 mutation and melatonin dosing, support the prediction that increasing senescence rate reduces cancer while reducing lifespan, and vice versa. Studies of environmental agents associated with increased cancer might be re-examined to find if there is an association with longevity increases, which may markedly alter our view of such agents. We also show that if an agent functions by slowing both senescence and carcinogenesis, longevity is increased while reducing cancer. Dietary restriction is the only known intervention that accomplishes this, but there may be others. *Toxicology and Industrial Health* 2002; **18:** 365–376.

Key words: *cancer; dietary restriction; longevity; melatonin; p53; senescence*

Introduction

In earlier work, human epidemiological data and mice bioassay data both indicate that cancer incidence rates flatten and reduce markedly if the person or animal lives sufficiently long: >80 years for humans and >800 days for BALB/c mice (Pompei and Wilson, 2001a and b; Pompei *et al.*, 2001). Although one is not entirely able to rule out under-reporting of cancer incidence at old age for humans (Doll, private communication), the weight of the human data and the corroborating mice data suggest that the turnover might be at least in part a real biological effect. Further, the human data suggest that incidence rates for all cancers, over incidences ranging a factor of 100, peak at approximately the same age (mean 85.0 years $\pm$ 3.7 SD for males and 84.5 $\pm$ 7.1 for females, Pompei and Wilson, 2001a), suggesting that the unknown biology is strongly related to age and applicable to all cancers. Accordingly a modelling investigation was conducted to learn more about the possible properties of this proposed biological effect.

Beginning with the Armitage–Doll (1954) multistage model for cancer incidence $I(t) = at^{k-1}$, derived as a fit to early 1950s cancer mortality data for age range 25–74, it is recognized that this model is only a first order approximation of the exact mathematics describing the modelled cellular steps to produce cancer, and is valid only for small values of incidence (Moolgavkar, 1978; Moolgav-

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kar *et al.*, 1999; Pompei and Wilson, 2001a). From the Armitage–Doll model an expression was derived by Pompei and Wilson (2001a) which resulted in adding a factor possibly representing cellular senescence, $(1-\beta t)$, producing the formula $I(t)=(\alpha t)^{k-1}(1-\beta t)$. Recognizing this formula as a Beta function $f(x)=\lambda t^{r-1}(1-x)$ over the interval $0\le x\le 1$, where $x=\beta t$, it has the mathematical interpretation $f(x)$ is the probability density function for the $(r-1)$th largest of r uniform random variables. This may be interpreted as the probability density function for achieving $(r-1)$ stages (cancer creation) without achieving the rth stage (cancer prevention). For clarity we denote the function derived from the Armitage–Doll multistage model with added senescence as the Beta-AD-senescence model.

Whereas the textbook Beta function $f(x)$ is assumed to integrate to one as a proper probability distribution function (pdf) should, the derived Beta-AD-senescence function $I(t)$ does not, and its integral varies over a range of about 0.002–0.526 for human cancers (Pompei and Wilson, 2001a). One possible interpretation is that a coefficient C, representing a susceptible subpopulation, might be applied for each cancer, as discussed in Pompei and Wilson. The major evidence against this idea is that susceptibility requires heterogeneity in the population, certainly reasonable for genetic and exposure differences for humans. However the 2234 undosed control mice of the ED01 study of Pompei *et al.* (2001) were a single inbred strain, carefully housed and maintained such that they were as little different from each other as possible. As these mice clearly did not all develop cancer simultaneously, but rather over their full lifetime range (distributed as a Beta-AD-senescence function), the data seem to support the long-standing idea that for equal genetics and exposure, cancer risk is still largely stochastic.

The Beta-AD-senescence function was shown to fit the human and mice data well, and thus might be considered a model. Figure 1 compares the fit of the Beta-AD-senescence function to data from four different sources compared to two historically important cancer models: the Armitage–Doll multistage, and the Moolgavkar–Vinson–Knudson (MVK) two-stage clonal expansion model (Moolgavkar and Knudsen, 1981). Figure 2 shows

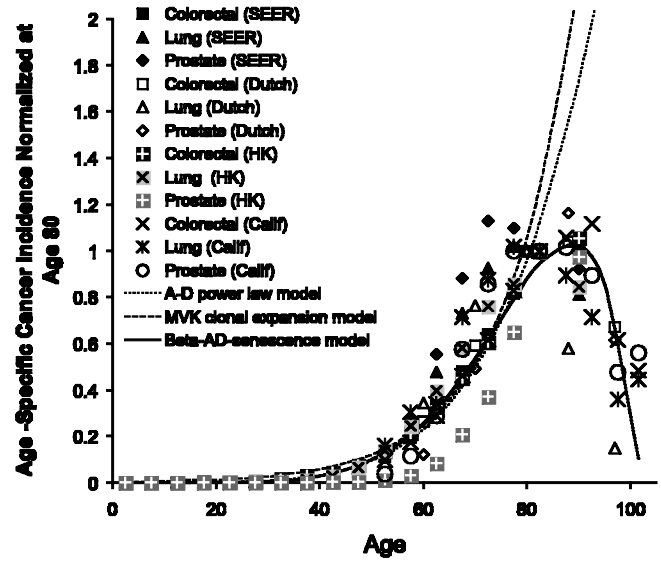

Figure 1. Age-specific cancer incidence as modelled by two historically important models: Armitage–Doll power law model and Moolgavkar–Vinson–Knudson clonal expansion model, compared to the Beta-AD-senescence model and data from four sources for three common cancers in males (SEER-USA, Holland, Hong Kong, California). Data is normalized to the value of incidence at age 82. Data from Pompei and Wilson (2001a).

the Beta-AD-senescence fit to data from the ED01 mice study. These data suggest a biological cause or causes not explained by previous models of cancer formation.

Several suggestions for this unknown biology were explored briefly in the earlier work, including cellular replicative senescence, which might be interpreted as a late stage cancer-limiting step.

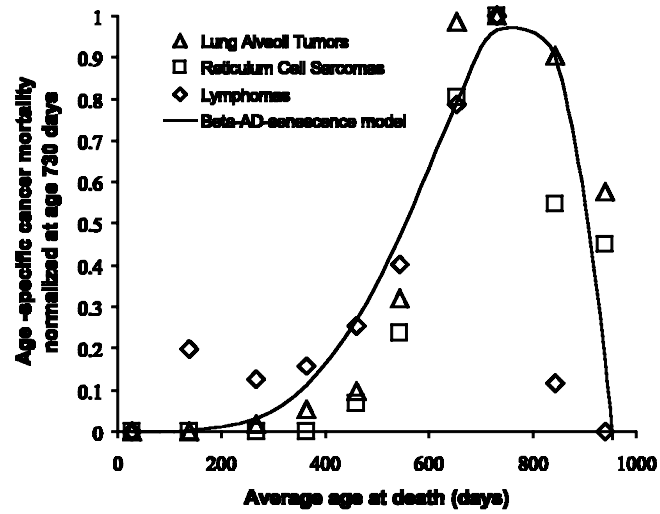

Figure 2. Age-specific cancer mortality. The three most common cancers for the ED01 mice study, normalized to the value of incidence at age 730 days, fitted with the Beta-AD-senescence model. Data from Pompei *et al.* (2001).

Senescence appears to be a good candidate, since it is widely accepted that: 1) cellular replicative capacity is limited; 2) this limitation has been observed *in vitro* and *in vivo*, both animal and human; 3) it is closely related to the ageing process; 4) it is a dominant phenotype when fused with immortal tumour-derived cells; 5) it is considered to be an important anti-tumour mechanism, since a senescent cell cannot produce cancer; 6) cells appear to senesce by fraction of population, rather than all at the same time; and 7) senescent cells continue to function normally, but are unable to repair or renew themselves (Pompei and Wilson, 2001b).

In addition to the characteristic peak in incidence occurring at about age 85, the Beta-AD-senescence model suggests, and both human and mice data appear to support, that cancer incidence for all organ sites might approach zero at an age approximating a natural human lifespan: 100.1 ± 8.2 years for the 40 SEER human male and female cancers (Pompei and Wilson, 2001a), and approximately 1000 days for BALB/c mice (Kodell *et al.*, 1980; Pompei *et al.*, 2001). We simply accepted at face value this apparent relationship between zero cancer incidence rate and end of lifespan: when cellular replicative capacity reaches zero (100% senescence), death from natural causes is near.

The Beta-AD-senescence function derivation is just one possible mathematical interpretation of the effect of senescence on cancer incidence, a result of adding senescence as a rate-limiting step to the multistage Armitage–Doll power law cancer model. To consider a second mathematical interpretation based on an entirely different but also highly successful cancer model, the often-used approximate form of the MVK two-stage clonal expansion model (Moolgavkar and Knudsen, 1981) may be modified to include senescence. The result, discussed below, is very similar to the Beta-AD-senescence model result, suggesting a robustness to the senescence interpretation.

The discovery of the now well accepted existence of cellular senescence is usually credited to Hayflick (Hayflick and Moorhead, 1961; Hayflick, 1965), who found that cells had finite and predictable number of doublings that can be achieved *in vitro*, and this limit might be directly related to ageing. Later investigators found that cells do not all reach their limit in population doublings simultaneously, but rather the number of nonreplicating cells gradually increases as a fraction of the total cells (Cristofalo and Sharf, 1973; Hart and Setlow, 1976; Dimri *et al.*, 1995; Campisi *et al.*, 1996; Rubelj and Vondracek, 1999; Campisi, 2000; Faragher, 2000; Rubelj *et al.*, 2000; Paradis *et al.*, 2001). That there is a relationship between cellular senescence and ageing has been firmly established (Campisi, 1997; 2000; 2001; Jennings *et al.*, 2000; Leung and Pereira-Smith, 2001; Paradis *et al.*, 2001; Tyner *et al.*, 2002). That senescence is an important tumour-suppressing mechanism is also well established (Sager, 1991; Campisi, 1997; 2000; 2001; Faragher, 2000).

Experimental evidence for increasing senescence with population doublings is shown in Figure 3, which suggests the fraction of cells senescing with population doublings is approximately linear (Hart and Setlow, 1976; Thomas *et al.*, 1997; Wynford-Thomas, 1999). There appears to be no evidence that the rate of senescence is related to the remaining fraction of proliferating cells, which would produce an exponential decay in the number of proliferating cells. Rather, there is a finite limit of doublings that any individual cell can achieve, as Hayflick had observed. That population doublings and *in vivo* age are linearly related is less easily observed, but the data of Figure 4, where *in vitro* observations of cells from a range of donor ages were conducted (Ruiz-Torres *et al.*, 1999; Yang *et al.*, 2001), suggest an approximately linear relationship.

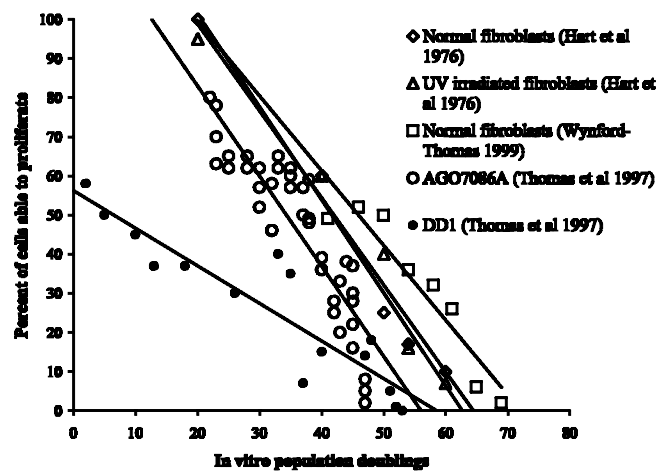

Figure 3. Cellular senescence evidence *in vitro*. Increase in number of population doublings decreases the number of cells which retain replicative capacity at an approximately linear rate. Lines indicate best linear fit for each data set.

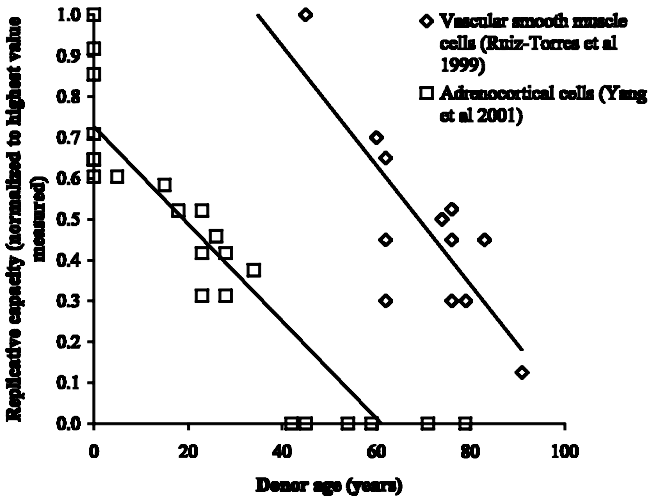

Figure 4. Cellular senescence evidence with increase in age of the donor. Increase in donor age decreases the number of cells which retain replicative capacity at an approximately linear rate. Lines indicate best linear fit for each data set.

A linear relationship between age and fraction of cells capable of proliferating implies that each individual cell has an approximately uniform probability of senescing per unit time, a stochastic process related to cellular damage, as suggested by many investigators (Reddel, 1998; Rubelj and Vondracek, 1999; Rubelj *et al.*, 2000; Duncan *et al.*, 2000; Toussaint *et al.*, 2000; Suzuki *et al.*, 2001). As suggested by Figure 4, the cumulative probability of senescence for any individual cell and for all cells, appears to reach one over a lifetime. There has been some controversy over this interpretation, however (Cristofalo *et al.*, 1998).

Cellular apoptosis is a related phenomenon to senescence as a cancer control mechanism by preventing damaged nonreparable DNA from reproducing. The difference is that apoptosis destroys the damaged cell, which is then replaced by a normal cell from a proliferating neighbour, while senescence leaves the cell in a functional state, but unable to reproduce (Faragher, 2000; Ran and Pereira-Smith, 2000; Campisi *et al.*, 2001). Senescence appears to be a much more common event than apoptosis, as recent experimental evidence with arsenic indicates a factor of 100 difference in rates (Liao *et al.*, 2001).

To explore whether senescence might be the hypothesized biological mechanism causing the turnover in cancer incidence at old age, we consider experiments which alter senescence in some way. Recent experiments with mice with genetically altered *p53* expression, and mice with long term melatonin dose, suggest altered senescence and might be studied. In addition, dietary restriction (DR) has long been known to significantly affect both cancer and longevity, and also might be altering senescence in some way.

The recent experiment by Tyner and colleagues (2002) with mice with altered *p53* shows that increased senescence is not only associated with markedly reduced cancer, but also markedly reduced longevity with extensive signs of premature aging. It is generally accepted that *p53* is an important tumour suppressor gene, since almost half of human cancers examined have mutated *p53* (Venkatachalam *et al.*, 1998). Further, a condition with an inherited mutated *p53* in one allele, known as the Li-Fraumeni syndrome, is known to cause cancer predisposition. A person with this syndrome will develop cancer with 50% probability by age 30 (Venkatachalam *et al.*, 1998). *p53* is also known to influence senescence (Bargonetti and Manfredi, 2002; Blagosklonny, 2002). The Tyner experiment stimulated us to examine the role of *p53* more closely.

A recent experiment by Anisimov *et al.* (2001), in which mice were dosed with melatonin for most of their lives, showed evidence of reduced senescence: increased longevity and increased cancer, and delayed signs of ageing. Melatonin is a naturally occurring hormone considered a chronobiotic, due to its association with circadian periodicity, both as a marker, and as an influence (Armstrong and Redman, 1991). Melatonin has been found to be protective against cellular oxidative damage (Beckman and Ames, 1998; Reiter, 1999), and influences senescence and ageing (Pierpaoli and Regelson, 1994). The Anisimov experiment led us to examine melatonin more closely.

Dietary restriction is known to simultaneously significantly reduce or postpone cancers while extending lifespan, a very different result than *p53* or melatonin intervention. DR has not previously been discussed in the context of senescence, but there is ample and long standing evidence in the literature that lifespan is extended with this intervention (Masoro *et al.*, 1982; Hansen *et al.*, 1999; Hart *et al.*, 1999; Sheldon *et al.*, 1995), and thus might have a significant effect on senescence. Further, it has been shown that cells retain the properties of the DR intervention in the whole

animal, when removed and cultured *in vitro*, suggesting a heritable alteration (Hass *et al.*, 1992). One long-held interpretation of the effect of DR is timescale stretching (Masoro *et al.*, 1982; Greenburg, 1999) for both longevity and cancer.

Methods

Data sources for modelling and model comparisons for possible variations in senescence in mice are from published work: 1) age-specific cancer mortality from the ED01 study of 24 000 female BALB/c mice (Pompei *et al.*, 2001); 2) effect of *p53* mutation on cancer mortality and longevity on genetically modified mice from Tyner *et al.* (2002); 3) effect of melatonin on cancer mortality and longevity on female CBA mice from Anisimov *et al.* (2001); and 4) effect of DR on longevity and cancer in seven rodent studies (Fernandes *et al.*, 1976; Masoro *et al.*, 1982; Weindruch *et al.*, 1982; 1986; Haseman, 1991; Seilkop, 1995; Sheldon *et al.*, 1995; Pompei *et al.*, 2001).

Designed to detect the effective dose of 2-acetylaminoflourene (2-AAF) required to produce 1% tumour rate, the ED01 controls' cumulative cancer mortality (including morbidity) was about the same as the Tyner wild type $p53^{+/+}$ (normal p53 in both alleles) cancer rates. Tyner produced mice with one *p53* allele mutated ($p53^{+/m}$) which the authors believe enhanced senescence, a third group with *p53* absent from one allele ($p53^{+/-}$) are believed to reduce senescence, and a fourth group with *p53* absent from both alleles ($p53^{-/-}$) which are believed to reduce senescence further. In modelling, experimental variation in senescence is computed from maximum longevity for the $p53^{+/+}$ and $p53^{+/m}$ groups, since lifetime was limited by causes unrelated to the cancers. For the $p53^{+/-}$ and $p53^{-/-}$ groups, senescence was arbitrarily taken as 0.5 of normal and 0 respectively. The senescence variations assumed to be caused by *p53* variations in Tyner are applied mathematically to the ED01 results, to compare the model results against the observations of Tyner.

Anisimov *et al.* (2001) dosed female CBA mice with 20 mg/L in drinking water for 5 consecutive days each month from age 6 months until their natural deaths, and compared cancer incidence, longevity, and physiological markers to undosed controls. The increase in longevity and increase in cancer is interpreted in the present work as caused by a reduction in senescence associated with melatonin. The relative senescence is computed from the maximum longevity ratio between dosed and control groups. The results are compared to the model predictions for altered senescence.

The senescence rate is modelled as the value β and has units of t^{-1}, where t is age. We make the simplest assumption throughout: that no cells are senescent at $t=0$ and all cells are senescent at $t=\beta^{-1}$. Normal senescence is taken as the value of β necessary to fit the cancer mortality data for normal mice, and corresponds to the inverse of the age at which modelled cancer incidence reaches zero. Relative senescence is modelled by the relative longevity of the mice compared to normal in the three experiments studied, when the longevity is not limited by cancers. Where cancer data is given as age-specific mortality, it is defined as animals dying of cancer in the time period, divided by the animal-days at risk. Since the age-specific mortality $M(t)$ is a hazard function (animals dying previously are not in the denominator), the cumulative probability of mortality is computed as $\text{Prob}=1-\exp[-\int M(t)\,dt]$. A model of longevity versus senescence is constructed by assuming death occurs at the age at which senescence reaches 100%, or the age at which age-specific cancer mortality reaches 80%, whichever occurs first. A model of probability of cancer mortality versus senescence is constructed by varying the value of β in the Beta-senescence model.

The Armitage–Doll model with senescence, denoted here as the Beta-AD-senescence model, is derived in Pompei and Wilson (2001a). The MVK model with senescence, denoted here as the Beta-MVK-senescence model, is derived from the commonly used approximate version $I(t)\approx \mu_1\mu_2\int N(s)\exp[(\alpha_2-\beta_2)(t-s)]\,ds$ (Moolgavkar and Knudsen, 1981). The integration is taken from 0 to t, μ_1 and μ_2 are the rates of the two transitions (initiation and malignancy), α_2 and β_2 are the growth and death rates of initiated cells respectively [$(\alpha_2-\beta_2)$ assumed positive], and $N(s)$ is a variable normal cell number function. For the simplest case of constant cell numbers, the integration yields: $I(t)=(N\mu/\gamma)[e^{\gamma t}-1]$ where $\gamma=(\alpha_2-\beta_2)$, and $\mu=\mu_1\mu_2$ and produces the curve indicated in Figure 5.

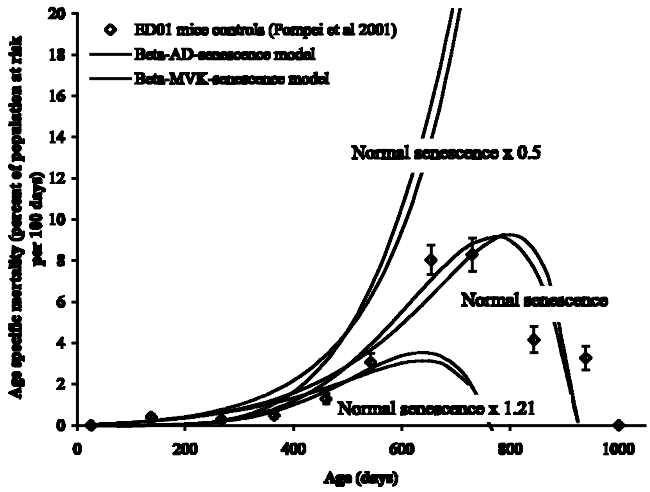

Figure 5. Influence of senescence rate on age-specific cancer incidence in mice. Beta-AD-senescence model fit to ED01 undosed controls is $I(t) = (\alpha t)^{k-1}(1-\beta t)$, where $\alpha = 0.00115$, $k-1 = 5$, $\beta = 0.00108$ (Pompei *et al.*, 2001). Equivalent Beta-MVK-senescence model fits shown. Senescence rate is the value of parameter β. Senescence rate increase by 21% is calculated from Tyner *et al.* (2002) results of 21% reduction in median lifespan for $p53^{+/m}$ mice compared to normal $p53^{+/+}$ mice. Senescence rate of 50% is an assumption for $p53^{+/-}$ mice of Tyner *et al*.

The simplest method of adding senescence is to assume it is a limiting stage with stage probability $(1-\beta t)$, yielding the relation $I(t) = (\mu/\gamma)[e^{\gamma t} - 1](1-\beta t)$ for the Beta-MVK-senescence model.

The time-stretching effect of DR is modelled by applying the assumption that t in the Beta-AD-senescence model $I(t) = (\alpha t)^{k-1}(1-\beta t)$, changes in proportion to caloric intake, or in proportion to weight, which is assumed to be a reasonable measure of caloric intake. To model total probability of tumours, the cumulative distribution function (cdf) is required, which is the integral of the Beta-AD-senescence function, and is denoted as $B(t) = (at)^k(1-bt)$, where $a = [\alpha/k^{1/(k-1)}]^{(k-1)/k}$ and $b = k\beta/(k+1)$; $0 \le t \le \beta^{-1}$. The cumulative distribution function $B(t)$ is similar to the Beta-AD-senescence function, but has different constants a and b in place of α and β, and exponent k instead of $k-1$. Since the value of t is still limited to β^{-1}, $B(t)$ never reaches a negative slope, ending at the peak value of probability with zero slope at $t = \beta^{-1}$.

Results

Figure 5 shows the result of varying the value of the senescence parameter β on age-specific cancer mortality, for both the Beta-AD-senescence and Beta-MVK-senescence models. The particular values of normal, 1.21 times normal, and 0.5 of normal were chosen to correspond to the senescence values calculated from the Tyner data, presented in further detail below. The ED01 controls data of Pompei *et al.* (2001) are taken as normal senescence data, to which both models are fit. As shown, the Beta-AD-senescence and Beta-MVK-senescence model curves are only slightly different in shape, and give essentially the same result with variation in β.

The cumulative probability of cancer resulting from variations in senescence is presented in Figure 6. As indicated, normal senescence is assumed for the p53-normal ($p53^{+/+}$) mice, 1.21 times normal for the p53-enhanced ($p53^{+/m}$) mice and 0.5 for the p53-deficient ($p53^{+/-}$) mice. The 1.21 value is calculated as the ratio of the median longevity of the $p53^{+/+}$ group compared to the $p53^{+/m}$ group, etc. As shown, the two approximate models produce cancer rates predictions which are in good agreement with the Tyner data. Although recently criticized as inadequate (Moolgavkar *et al.*, 1999), we found the approximate form of the MVK two-stage clonal expansion model adequate for our purposes.

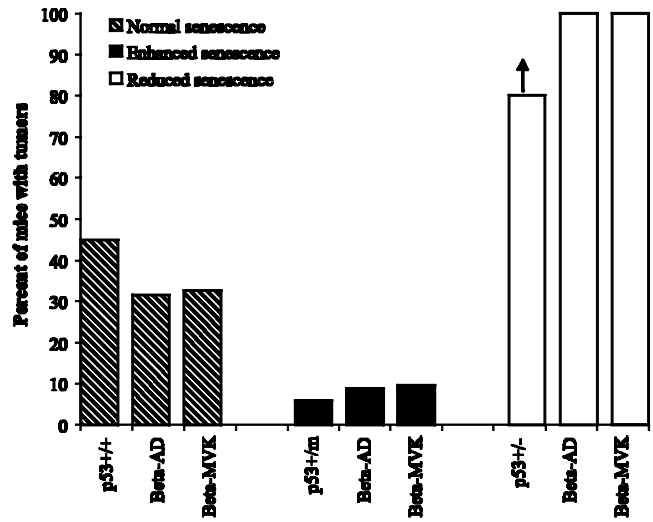

Figure 6. Probability of tumours in Tyner *et al.* (2002) compared to Beta-AD-senescence and Beta-MVK-senescence models predictions. Modelled lifetime probability of cancer is calculated as $\text{Prob} = 1 - \exp[-\int M(t)\,dt]$, where $M(t)$ is age specific mortality. Tyner *et al.* results for $p53^{+/+}$, $p53^{+/m}$, and $p53^{+/-}$ are interpreted as normal senescence, 21% enhanced senescence, and 50% reduced senescence respectively. Arrow indicates Tyner data reported as $>80\%$ tumour rate.

Anisimov *et al.* (2001) data for mice dosed with melatonin are shown in Figure 7 as age-specific mortality versus age. There is a marked difference in the curves between dosed and controls, with the controls showing turnover, and the dosed with no turnover. However, since there were only 50 mice in each group, three deaths by tumour in the controls, and 13 deaths by tumour in the dosed group, the error bars are large. Anisimov *et al*. report that the difference in cancer mortality between the two groups is statistically significant ($P < 0.001$).

Cancer mortality and lifetime versus senescence rate are shown in Figure 8, combining the data from all of the sources and comparing them to Beta-AD-senescence model predictions. The predictions for cancer are the direct calculation of cumulative cancer mortality versus normalized senescence rate. The predictions for lifetime are the lesser of the age at which $t = \beta^{-1}$ (age at which cancer incidence drops to zero), or cancer age-specific mortality reaches $> 80\%$, the reported cancer rate by Tyner for $p53^{+/-}$ mice. The lifetime prediction curve shows a peak value of about 1.3 at a value of 0.75 for normalized β. As shown, cancer mortality follows the model prediction's trends, with cancer rates approaching zero at senescence value > 1.2, and approaching certainty at senescence < 0.6. The lifetime data follow the model predictions for senescence > 0.8, since these points were used to 'calibrate' the value of senescence (lifetime ends by reaching 100% senescence). For senescence < 0.8, the model departs from the data for lifetime limited by cancer mortality, and a curve fit is shown for clarity. Human (SEER) cancer mortality is shown for comparison.

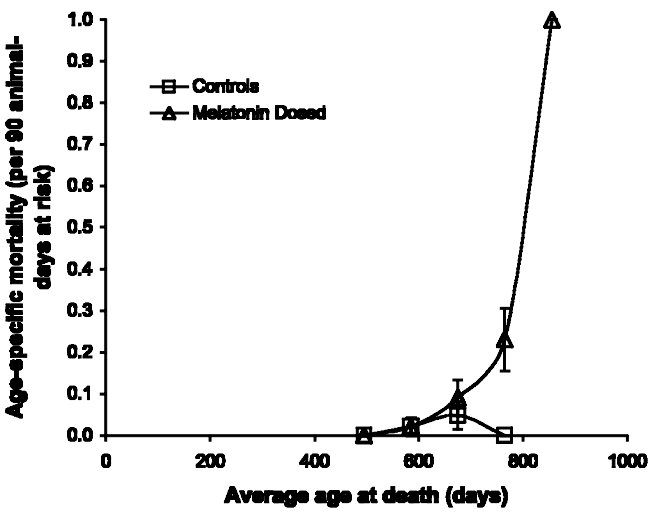

Figure 7. Age-specific cancer mortality for female CBA mice dosed with melatonin versus controls. Data from Anisimov *et al.* (2001).

Figure 9 shows the results of two investigations into the relationship between weight and mice liver tumours from the National Toxicology Program (NTP) database. A Beta-AD-senescence model fit is shown for comparison, where the fit is developed by varying time t in proportion to weight while holding all other variables constant, in accordance with the interpretation that DR stretches time. The assumption is made that weight is a reasonable approximation to caloric intake.

Figure 10 shows the results of five rodent studies of the effect of DR on mean lifespan. The Beta-AD-senescence model comparison line is computed by holding all variables constant while varying t in inverse proportion to caloric intake. These data suggest that the model can be fit accurately by adding only a coefficient of about 0.9 to the inverse proportionality, suggesting that about 10% of the causes of death might be attributable to unrelated mechanisms.

Discussion

The central hypothesis of this work is that the turnover observed in age-specific cancer incidence, as illustrated by Figures 1 and 2, is caused by increasing cellular replicative senescence: as age increases, fewer cells are available to become cancerous because only nonsenescent cells retain proliferative ability. Figures 3 and 4 show seven examples of generally accepted *in vitro* data supporting the reduction in the number of proliferating cells with age. A linear senescence versus age assumption leads to the $(1 - \beta t)$ factor added to the Armitage–Doll multistage power law model, which can be interpreted as a limiting last stage, becoming the Beta-AD-senescence model $I(t) = (\alpha t)^{k-1}(1 - \beta t)$ used to successfully fit human and mice age distribution cancer data including the turnover at old age. The senescence hypothesis may also be applied to the two-stage clonal expansion MVK model with the same result, as shown in Figure 5.

A further test of the senescence hypothesis is to compare the model predictions to available data on animals which have been subjected to treatment

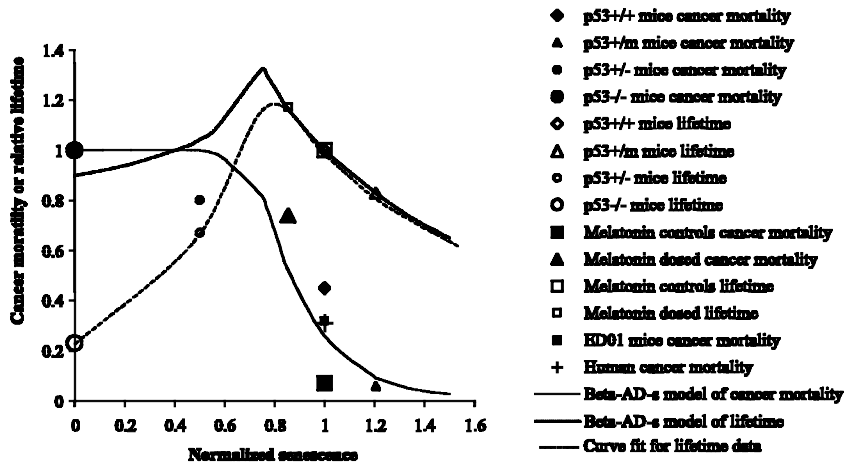

Figure 8. Influence of senescence rate on cancer mortality and lifetime: data from Tyner *et al.* (2002) for mice with $p53^{+/+}$, $p53^{+/m}$, and $p53^{+/-}$; compared to Beta-AD-senescence model predictions. Beta model predictions for cancer mortality are $\text{Prob} = 1 - \exp[-\int M(t)\,dt]$. Beta model predictions for lifetime are calculated as the lesser of: age at which senescence reaches 100% ($t = 1/\beta$), or age at which age-specific cancer mortality reaches 80% [$M(t) = 0.8$]. Human cancer mortality computed from SEER data.

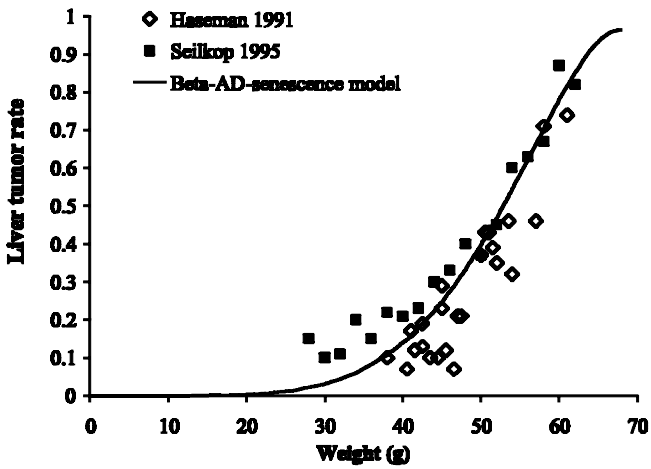

Figure 9. Liver tumour incidence versus weight for two studies of control female B6C3F1 mice. Seilkop data based on body weight measured at 12 months, Haseman data based on maximum weekly average weight. The Beta-AD-senescence model fit was developed by varying t in proportion to weight.

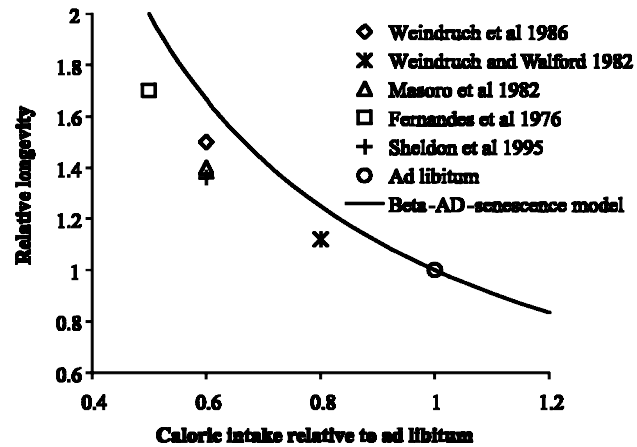

Figure 10. Results of five rodent studies of the effect of DR on mean lifespan. The Beta-AD-senescence model comparison line is computed by varying t (or equivalently β) in inverse proportion to caloric intake.

which may be altering senescence in some way. As *p53* is known to induce senescence, the Tyner *et al.* (2002) experiment with genetically altered mice showed that increased *p53* activity, which leads to increased senescence, results in shorter lifespan (with extensive symptoms of premature aging) but decreased cancers. With mice with 50% reduced senescence (assumed, with one allele missing *p53*), cancers increased substantially and directly caused shorter lifespan by cancer mortality. Mice with senescence reduced to zero (assumed, with both alleles missing *p53*), lifespan decreased still further due to even earlier onset of lethal cancers. The comparisons with the Beta-AD-senescence and Beta-MVK-senescence model predictions of Figure 6 suggest that the main features of the proposed senescence hypothesis on cancer are well supported by the *p53* data.

Melatonin is not usually considered a modifier of senescence, but in addition to its chronobiotic properties, is well known as an antioxidant that reduces damage to DNA. It is through its damage protective properties that the action of melatonin might be interpreted to influence senescence, since oxidation damage is known to be a cause of senescence. The experiment of Anisimov *et al.* (2001) resulted in melatonin-dosed mice exhibiting maximum longevity 17% longer than controls, but with five times the lethal tumours than controls,

although total tumours were about the same (22 versus 20). The age distribution of mortality due to cancer plotted in Figure 7 shows the same features as the model predicts in Figure 5: normal senescence results in turnover in cancer, while reduced senescence eliminates the turnover.

Ferbeyre and Lowe (2002) observed that there is a balance between cancer and ageing in their commentary on the Tyner paper, sketching a curve of lifespan versus *p53* activity, wherein the curve shows lifetime peak at normal *p53* activity. With the Beta-senescence model we quantify such a curve, and compare the model to the data. These results, shown in Figure 8, confirm Ferbeyre's observation that there ought to be a peak, but also suggest the intriguing possibility that a longevity peak is higher than normal for lower values of senescence than normal: about 1.3 times normal longevity at 0.75 of normal senescence. This results from accepting higher levels of cancer as the cost of longer life, an attractive possible strategy if many cancers can be successfully treated by modern medicine.

The left part of the lifetime data of Figure 8 drops considerably more rapidly than predicted by the model, which raises questions about the validity of some of the assumptions. The low senescence data is entirely based on the assumption that reduced *p53* reduces longevity only by increasing cancer, which in turn occurs only because of reduced senescence. This is clearly a gross simplification, since *p53* is known to be very important in DNA repair as well as causing apoptosis, both of which affect cancer rate without necessarily involving senescence. A second gross simplification might be the cancer creation assumptions represented by the Armitage–Doll multistage and MVK clonal expansion models, since these formulations were based on biological assumptions that did not include senescence, but data that they were fit to, did. It is instructive to consider the aforementioned Li-Fraumeni syndrome, which causes cancer with 50% probability by age 30, a difficult point to reconcile with either model, even with the removal of senescence.

The link between cancer and longevity, which appears to be a cardinal characteristic of senescence, leads to testable hypotheses. One possibility is the activity of arsenic, a known human carcinogen at high doses, but recently shown to be a strong inducer of senescence *in vitro* (about 100 times the rate of apoptosis induction), which might be a reason arsenic rarely exhibits carcinogenicity in animal models (Liao *et al.*, 2001). Accordingly, an epidemiological study of longevity versus low levels of arsenic ingestion might show both longevity reduction and cancer reduction, as predicted by Figure 8. Similarly, epidemiological studies on many environmental or diet influences that might include longevity data with cancer data might be re-examined to find if the expected correlations are observed.

A confounding effect on cancer rate might be the possible action of antioxidants to directly reduce cancers by reducing DNA damage (Beckman and Ames, 1998). However, studies have shown that this is not a consistent result, and dietary supplementation may increase cancer (Potter, 1997). It is possible that observations of increased cancer with antioxidant supplementation might be due to the action of the antioxidant in reducing senescence. The issue might be settled in such studies by longevity data. Of particular interest are agents that might reduce damage to DNA sufficiently to both increase longevity and reduce cancer, a combination so far observed most clearly for DR (Hart *et al.*, 1999; Roth *et al.*, 2001).

That DR intervention may alter senescence, perhaps through time stretching, but has not yet been directly measured by *in vitro* studies of cells taken from DR donors compared to *ad libitum* donors. However, the comparisons between the Beta-AD-senescence model results, and cancer and longevity data of Figures 8 and 9, provide support for this model interpretation. TDMS data reported in Pompei *et al.* (2001) is not modelled, but appears to support the idea that DR might stretch time, as it relates to carcinogenesis.

DR intervention creates very complex biochemical responses, and most but not all of them are consistent with the time-stretching hypothesis. As noted by Anisimov (2001): 'It was calculated that 80–90% from 300 various parameters studied in rodents maintained on the calorie restricted diet (including behavioural and learning capacity, immune response, gene expression, enzyme activity protein synthesis rate, effects of hormones, glucose tolerance, DNA repair efficacy) revealed features of

slow aging.' Accordingly, the alternative Beta-AD-senescence model interpretation that α and β vary in proportion to caloric intake, may be a more precise interpretation. This suggests that DR increases longevity by decreasing the rate of senescence β, and simultaneously reduces cancer by reducing the rate of each stage of carcinogenesis, as represented by the value of α, by the same proportion. Further evaluation of this alternative to time stretching will have to await exact models of carcinogenesis derived with senescence, and more extensive data to test such models.

Dietary restriction is the only consistently effective intervention we know of that both increases longevity and reduces cancer, but there may be others. For example, selenium has shown some promise in this regard in certain experiments (Anisimov, 2001). In searches for life-extending interventions, clearly those similar to DR are the most desirable. The characteristics to be sought are reduction or slowing of damage to DNA which causes both carcinogenesis and senescence.

Our work suggests that if we live long enough, cellular replicative senescence might allow us to outlive our cancers, but inevitable ageing caused by that senescence will make these cancer-free years relatively limited. The strategy of reducing senescence via some intervention appears to have the side effect of increased cancer, but this might be an acceptable cost given our reasonable and improving success at treating cancers. Also, there might be some interventions, such DR, which might accomplish both reduction in senescence and reduction in cancer. Accordingly we might consider the following future work:

1) Toxicological and carcinogenicity rodent bioassays designed to last the full natural lifetime instead of the standard two years, in order to build a data base to study the cancer rate turnover and longevity, features that are missing from the available large databases.
2) Re-examine studies for various dietary or environmental influences for effects on longevity as well as cancer. Various studies have been performed on the effect of various agents or actions on cancer: antioxidants, DR, pollutants, dietary supplements, and others. These all address in some way damage to DNA that might cause cancer, either preventing it in the case of a presumed antioxidant agent for example, or causing it in the case of a carcinogenic agent. However this paper, especially the data on *p53* and melatonin, shows that these studies are incomplete unless they also address the issue of longevity. A really useful anti-cancer agent will both increase longevity and decrease cancer.
3) Exact mathematical modelling of cancer mechanisms with senescence. An exact form of the Armitage–Doll multistage model would take into account the fact that if the later transition stages proceed quickly, the number of cells which are available for proceeding to cancer will be affected. This depletion of cells available to proceed to later stages of cancer is common to several biological effects, including senescence. It is an important next step to perform precise mathematical modelling to include all possibilities of reduction of the pool of cells, including by senescence; slowing of biological processes at older ages; and effects on biological processes by DR. As part of our ongoing modelling work, Dr Dmitri Burmistrov (private communication) has recently suggested an alternate interpretation of the extra term $(1 - \beta t)$. If all cells, at whatever stage of the cancer formation and growth process, have a probability of becoming senescent at any time, the extra term will appear.

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