p53 mutations associated with aging-related rise in cancer incidence rates

Richard B Richardson

Radiological Protection Research and Instrumentation Branch; Atomic Energy of Canada Limited; Chalk River Laboratories; Chalk River, ON Canada

TP53's role as guardian of the genome diminishes with age, as the probability of mutation increases. Previous studies have shown an association between p53 gene mutations and cancer. However, the role of somatic *TP53* mutations in the steep rise in cancer rates with aging has not been investigated at a population level. This relationship was quantified using the International Agency for Research on Cancer (IARC) *TP53* and GLOBOCAN cancer databases. The power function exponent of the cancer rate was calculated for 5-y age-standardized incidence or mortality rates for up to 25 cancer sites occurring in adults of median age 42 to 72 y. Linear regression analysis of the mean percentage of a cancer's *TP53* mutations and the corresponding cancer exponent was conducted for four populations: worldwide, Japan, Western Europe, and the United States. Significant associations ($P \le 0.05$) were found for incidence rates but not mortality rates. Regardless of the population studied, positive associations were found for all cancer sites, with more significant associations for solid tumors, excluding the outlier prostate cancer or sex-related tumors. Worldwide and Japanese populations yielded P values as low as 0.002 and 0.005, respectively. For the United States, a significant association was apparent only when analysis utilized the Surveillance, Epidemiology, and End Results (SEER) database. This study found that *TP53* mutations accounts for approximately one-quarter and one-third of the aging-related rise in the worldwide and Japanese incidence of all cancers, respectively. These significant associations between *TP53* mutations and the rapid rise in cancer incidence with aging, considered with previously published literature, support a causal role for *TP53* according to the Bradford-Hill criteria. However, questions remain concerning the contribution of *TP53* mutations to neoplastic development and the role of factors such as genetic instability, obesity, and gene deficiencies other than *TP53* that reduce p53 activity.

Introduction

The *TP53* gene encoding the p53 protein has been called the guardian of the genome[1] because of its role in mediating tumor suppression by irreversible cell cycle arrest (replicative senescence) and apoptosis of cells with damaged DNA. However, *TP53* is the most commonly lost gene function in human cancers, with the great majority (>98%) of *TP53* gene mutations being acquired somatically.[2] These *TP53* mutations are chiefly point missense substitutions (75%), with other frequent alterations including frameshift insertions and deletions (9%), nonsense mutations (7%), and silent mutations (5%).[3] The effects of these mutations are complex, as in addition to decreased tumor suppression and depressed policing of oncogenic signaling, the loss of *TP53* may under some circumstances aid oncogenesis by endowing cancer stem cells with a proliferative (non-quiescent) and immortal gain of function.[4-7]

The mutational inactivation of *TP53* and its effect on the neoplastic process is also complex and depends on the grade at which the *TP53* mutation occurs. Mutations early in the process aid the transformation from benign stage to malignant form, leading to the Warburg effect and reliance on glycolysis. Later alterations of *TP53* can influence the transition from a non-invasive lesion to an invasive one.[8] Two animal studies published in 2007[9,10] determined that *TP53* mutations promote not only tumor development, but also established tumors. Furthermore, these experiments demonstrated that *TP53* restoration reversed the neoplastic process:

A

Incidence and Mortality

B

Incidence, Log-Log Plot
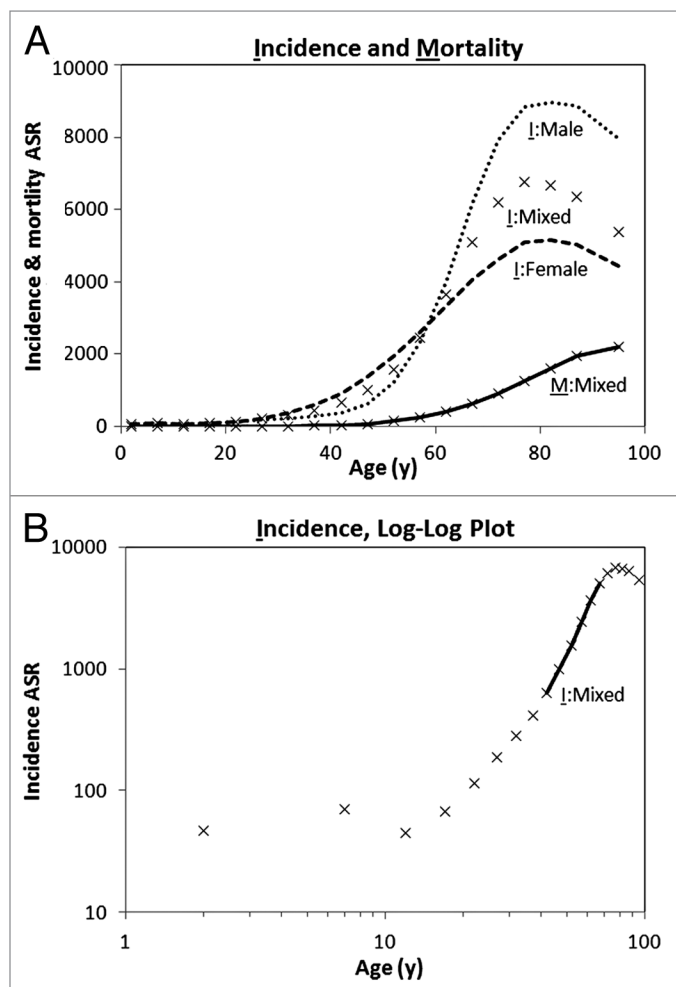
Figure 1. Annual incidence and mortality ASR of all cancers. (**A**) Incidence in 2007 (I) and average mortality in 2004–2008 (M) rates per 100 000 population (Statistics Canada)[14] for males, females, and both sexes (mixed). (**B**) Log-log plot of cancer incidence ASR and age for both sexes combined, showing the cancer exponent trend line.

Ventura et al.[9] showed that lymphoma cells underwent apoptosis and sarcomas displayed replicative senescence, while Xue et al.[10] found that liver tumor cells cleared rapidly, with apparent activation of the innate immune system. Surprisingly, not all mutations in *TP53* are inactivating; for example, abnormal accumulation (overexpression) of p53 is found in a few cancers, e.g., Hodgkin disease and breast cancer.[11,12] Furthermore, one study found that p53 can exert pro- or anti- apoptotic effects, depending on cellular context.[13]

Cancer incidence rates in adults typically rise steeply with age, with the increased incidence starting in the reproductive period and decelerating or declining in old age, about 70 y and over (**Fig. 1A** based on Canadian data).[14] Mortality rates also show an age-dependent trend similar to incidence rates but shifted by a time lag between the age at clinical manifestation and death from the disease (**Fig. 1A**). For many types of cancer, the incidence and mortality age-standardized rate (ASR; typically a weighted mean of age-specific rates for 100 000 of a standard population) among

adults (excluding the very old) can be represented by a power function:

$$ASR(t) = c\, t^K \quad (1)$$

where t is the age in years, K is the "cancer exponent", and c is a constant. A plot of $\log(ASR)$ vs. $\log(t)$ is a straight line with a slope of K (**Fig. 1B**):

$$\log(ASR) = \log(c) + K \log(t) \quad (2)$$

Armitage and Doll[15] hypothesized that a discrete number of mutational stages could be estimated from a rounded value of the cancer exponent plus 1. SRT such as those of the prostate, ovary, and uterus exhibit variable hormone and metabolism dependence and can depart from the power function. Breast tumors, for example, are initially dependent on estrogens or obesity-related metabolism for growth and progression; their incidence can be much reduced after menopause in populations that have not adopted a western-like lifestyle.[16,17]

TP53 is the subject of nearly 300 000 publications,[18] yet a quantitative estimate of the contribution of *TP53* mutations to cancer incidence and mortality is lacking. This study evaluates the role of somatic *TP53* mutations on the steep aging-related rise in regional cancer rates employing large, international, population-based databases. It first provides a quantitative analysis of the rise of adult cancer rates with age to determine whether there are significant associations with somatic *TP53* mutations in those cancers and, second, considers whether these associations, in combination with the evidence of published studies, support a causal role.

Results

Prevalence of *TP53* mutations in cancers

On the basis of IARC GLOBOCAN and *TP53* databases, *TP53* mutations are present in an estimated 29% of all cancer cases diagnosed globally (n = 25, ~92% of total incidence ASR), and 32% of solid tumor cases, i.e., excluding leukemia and multiple myeloma (n = 23, ~88% of total incidence ASR). When hormone-associated SRT (~27% of total incidence ASR) are excluded, a similar *TP53* mutation rate of 29%, and slightly greater rate of 34%, is found in all cancer cases and solid tumor cases diagnosed, respectively. Therefore, the mutations are found in about 1 in 3 of all malignant tumors occurring in the world population.

Regional relationship between *TP53* mutations and cancer incidence and mortality

Worldwide

For global data, the 27 cancer exponent (slope of log ASR vs. log age plot) values varied from –1.7 (testicular cancer) to 11.2 (prostate cancer) for incidence ASR and from 1.5 (cervix uteri cancer) to 11.0 (prostate cancer) for mortality 8ASR (**Table 1**). These 27 cancer exponents were obtained from linear regression plots whose mean R value was 0.97 ± 0.07 SD. For only 4 cancer sites (Kaposi sarcoma, nasopharynx, cervix uteri, and testis cancer) was the relationship weaker than $R < 0.95$ (**Table 1**).

For the mortality ASR, for all cancers and for solid tumors (both sexes or male and female separately), including and excluding SRT, regression analysis yielded no significant associations

($P > 0.05$) between the percentage of samples with *TP53* mutations and cancer exponents.

The same regression analyses were conducted separately for incidence ASR for males and females. Prostate cancer (~8% of total incidence ASR) was an outlier (standardized residual ≥3.0), with its highest cancer exponent (11.2) nearly double the next highest. This extremely high exponent may be related to benign nodular prostatic hyperplasia being found in almost all men after 50 y.[19] A significant association ($R^2 = 20\%$, $P = 0.03$) was found for females, which included 4 SRT, yet non-significant for males ($R^2 = 6\%$, $P = 0.3$), unless prostate cancer was excluded ($R^2 = 23\%$, $P = 0.03$; **Table 2**). Hence, the regression model indicates by R^2 that 20% or more of the cancer exponent variability is explained by *TP53* mutations.

For all cancers or solid tumors only, of both sexes combined, associations between *TP53* mutation rates and cancer exponents were non-significant if OPC was included yet significant if OPC was excluded ($R^2 = 24\%$, $P = 0.01$, all cancers, **Fig. 2A** and $R^2 = 38\%$, $P = 0.002$, solid tumors). Similarly, *TP53* mutation rates and cancer exponents were non-significant if SRT were included, yet significant for solid tumors only if SRT were excluded ($R^2 = 17\%$, $P = 0.08$, all cancers and $R^2 = 31\%$, $P = 0.02$, solid tumors).

Major cancer types originate as epithelial cells (carcinomas), hematopoietic cells (leukemia, lymphoma, myeloma), germ cells (testicular cancers), and mesenchymal cells (sarcomas). I compared *TP53* mutations in 20 primarily epithelial tumor sites and 4 non-epithelial tumor sites (leukemia, multiple myeloma, non-Hodgkin lymphoma, testis), excluding brain cancers, which have exceptional morphologic diversity, and Hodgkin lymphoma, which has less than 50 *TP53* samples. A highly statistically significant difference (t test, $P = 0.002$; $P = 0.0002$ including Hodgkin lymphoma) was found, with *TP53* mutation rates of 30 ± 12% SD in epithelial tumors and 12 ± 6% SD in non-epithelial tumors.

Japan, Western Europe, and United States
Similar to worldwide data, no significant link was identified between *TP53* mutations and mortality cancer exponents in each of these three regions. Prostate cancer was an outlier for Japan and Western Europe but not for the United States (standardized residual ~2.6). For all 3 regions, relationships between the *TP53* mutation rates and incidence cancer exponents for all cancers and solid tumors were non-significant when OPC or SRT were included.

For Japan, when OPC was excluded there was a stronger and highly significant association for all cancer sites ($R^2 = 38\%$, $P = 0.006$) and solid tumors, **Figure 2B** ($R^2 = 41\%$, $P = 0.005$). However, when SRT were excluded, there was a weaker yet significant association ($R^2 = 34\%$, $P = 0.03$) for all cancer sites and an association, although not quite significant ($R^2 = 29\%$, $P = 0.06$), for solid tumors.

For Western Europe, the reverse was true for SRT exclusion, with a weakly significant association ($R^2 = 28\%$, $P = 0.04$) for solid tumors and a non-significant association ($R^2 = 18\%$, $P = 0.09$) for all cancers. When OPC was excluded for Western Europe there was a significant association for solid tumor sites
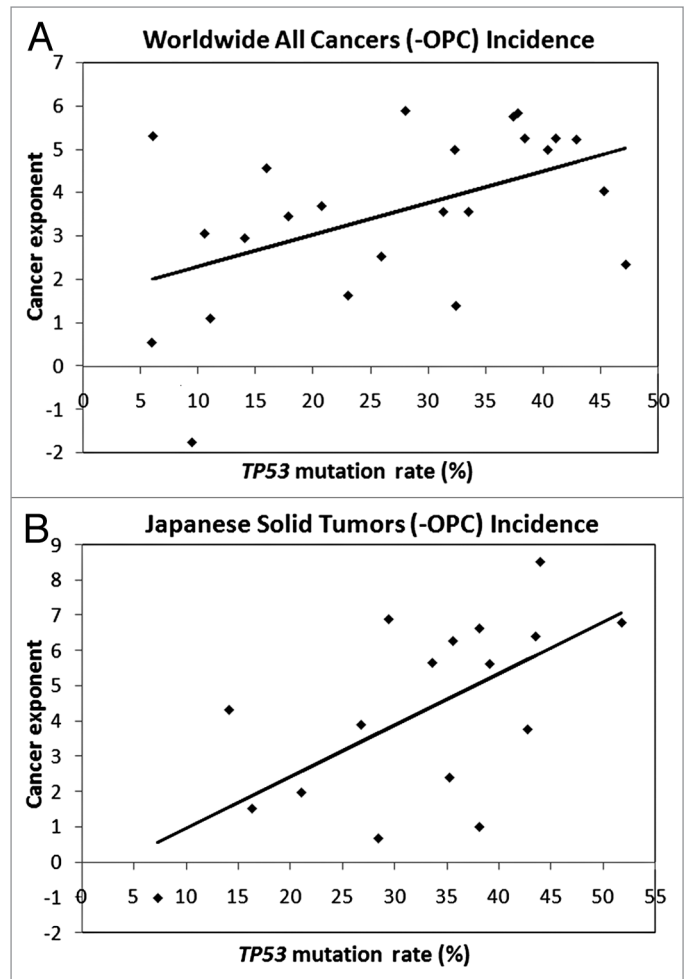
Figure 2. Linear regression trend lines for IARC incidence ASR and IARC *TP53* mutation rates for individuals aged 40–44 y to 65–69 y. (**A**) the worldwide incidence of 24 all cancers excluding OPC, $R = 0.49$, $CI = 0.11$–0.75, $P = 0.01$. (**B**) The Japanese incidence of 17 solid tumors, excluding OPC, $R = 0.64$, $CI = 0.24$–0.86, $P = 0.005$.

only ($R^2 = 16\%$, $P = 0.07$ all cancers, $R^2 = 24\%$, $P = 0.03$ solid tumors).

Similar regression analyses for all cancers and solid cancers utilizing IARC GLOBOCAN United States data showed no evidence of an association ($P \geq 0.2$). Prostate and breast cancer had the major standardized residuals (~2.7 and –1.4, respectively). Of the three regions, IARC data for the United States had the smallest number of all cancer and solid tumor sites with an adequate number of *TP53* samples (17 and 15 sites, respectively). I therefore employed the SEER cancer data set to increase the number of sites with adequate IARC *TP53* sampling data for all cancers and solid tumors (20 and 17 sites, respectively). In this case, a weakly significant association ($R^2 = 30\%$, $P = 0.05$) was found for the log-log plot of the percentage of samples with *TP53* mutations and cancer exponents for solid tumors when SRT were excluded.

The linear regression analyses of the *TP53* mutation rates and cancer exponents of all cancers and solid tumors, with or without SRT, were compared for paired geographical regions

Table 1. Analysis of worldwide cancer exponent values for IARC GLOBOCAN incidence age-standardized rates of cancer (6 five-year age groups; both sexes except for SRT) and IARC TP53 mutation rates, for various types of cancer by topographic or morphologic sites

Cancer site	TP53 cancer site (short title)	ICD-10	TP53 mutations %	Cancer exponent		R
				Incidence	Mortality	Incidence
Bladder[a]	Bladder	C67	28.0	5.9	6.8	1.00
Brain, nervous system[a]	Meninges, brain, spinal cord	C70–72	26.0	2.5	3.1	1.00
Colorectum[a]	Colon; colorectum NOS, rectosigmoid junction, rectum, anus	C18–21	42.9	5.2	5.6	1.00
Gallbladder[a]	Gallbladder, biliary tract[c]	C23–24	44.6	5.2	5.4	1.00
Hodgkin lymphoma	Hodgkin lymphoma[d]	C81	12.1[f]	1.2	2.5	0.97
Kaposi sarcoma	Not available (NA)	C46	NA	−1.2	NA	0.71
Kidney[a]	Kidney, renal pelvis, ureter	C64–66	15.9	4.6	5.1	1.00
Larynx[a]	Larynx	C32	40.4	5.0	4.6	0.99
Leukaemia[b]	Leukemias NOS[d], lymphoid leukemias, myeloid leukemias, other leukemias	C91–95	10.6	3.1	3.9	0.99
Lip, oral cavity[a]	Lip, tongue (base), tongue (other), gum, mouth (floor), palate, mouth (other), parotid gland, salivary glands	C00–08	33.5	3.6	3.4	0.99
Liver[a]	Liver	C22	31.3	3.6	3.5	0.99
Lung[a]	Trachea[e], lung	C33–34	37.4	5.8	5.8	1.00
Melanoma of skin[a]	Nevi and melanomas[d]	C43	14.1	2.9	3.9	1.00
Multiple myeloma[b]	Plasma cell tumors[d]	C88, C90	6.1	5.3	5.8	1.00
Nasopharynx[a]	Nasopharynx	C11	32.4	1.4	2.2	0.92
Non-Hodgkin lymphoma	Mature T- and NK-cell lymphomas[d], mature B-cell lymphomas, precursor cell lymphoblastic lymphomas	C82–85,C96	20.8	3.7	4.3	1.00
Esophagus[a]	Esophagus	C15	41.1	5.3	5.2	0.99
Other pharynx[a]	Tonsil, oropharynx, pyriform sinus[e], hypopharynx, other head and neck	C09–10,C12–14	45.3	4.0	3.2	0.98
Pancreas[a]	Pancreas	C25	37.8	5.8	5.9	1.00
Stomach[a]	Stomach	C16	32.3	5.0	5.3	1.00
Thyroid[a]	Thyroid	C73	11.1	1.1	4.7	0.95
Sex-related						
Breast[a]	Breast	C50	23.0	1.6	1.9	0.97
Cervix uteri[a]	Cervix uteri	C53	6.0	0.6	1.5	0.80
Corpus uteri[a]	Corpus uteri	C54	17.9	3.5	4.5	0.95
Ovary[a]	Ovary	C56	47.2	2.3	3.1	0.97
Prostate[a]	Prostate	C61	17.5	11.2	11.0	1.00
Testis[a]	Testis	C62	9.5	−1.7	2.3	0.93

[a]Invasive; [b]non-solid tumors; [c]included as only ~5–10% of bile duct cancers are intrahepatic; [d]site by morphology rather than topography; [e]no TP53 samples in this category; [f]<50 TP53 samples.

(Japan:Western Europe; Western Europe:United States; and Japan:United States) by ANCOVA. For every regression model, slopes and means did not differ significantly between paired regions. Similarly, after exclusion of prostate cancer, only the slopes for Japan:United States differed at a level that was close to significance or weakly significant ($P = 0.04$ all cancers, $P = 0.07$ solid tumors). These results indicate that the rise in cancer incidence with aging is a similar function of the TP53 mutation rate regardless of geographical region, except that TP53 mutations appear to have a greater influence on cancer rates in Japan than the United States.

Relationship between TP53 mutation rates and cancer incidence by country

Table 2. Regression parameters for cancer exponents from incidence ASR for all cancers and solid tumors and *TP53* mutation rates for individuals aged 40–44 y to 65–69 y, analyses conducted worldwide and in three geographical regions (IARC GLOBOCAN data with addition of SEER data for the United States)

Region	Cancer site group (both sexes unless otherwise stated)	Excluding OPC or SRT?	Cancer sites *n*	Slope	Intercept	R (95% CI)	R² %	P
World	Female, all	---	23	0.05	2.1	0.45 (0.04-0.72)	20	0.03[a]
	Male, all	---	21	0.05	3.1	0.26 (−0.20-0.62)	6	0.3
		-OPC	20	0.07	2.0	0.48 (0.04-0.76)	23	0.03[a]
	All	---	25	0.05	2.4	0.29 (−0.12-0.62)	8	0.2
		-OPC	24	0.07	1.6	0.49 (0.11-0.75)	24	0.01[a]
		-SRT	19	0.05	2.7	0.41(−0.05-0.73)	17	0.08
	Solid tumors	---	23	0.07	1.8	0.35 (−0.07-0.67)	12	0.1
		-OPC	22	0.10	0.6	0.62 (0.27-0.83)	38	0.002[a]
		-SRT	17	0.08	1.7	0.56 (0.10-0.82)	31	0.02[a]
Japan	All	---	19	0.08	2.3	0.30 (−0.18-0.66)	9	0.2
		-OPC	18	0.13	0.2	0.62 (0.21-0.84)	38	0.006[a]
		-SRT	14	0.09	2.1	0.58 (0.07-0.85)	34	0.03[a]
	Solid tumors	---	18	0.08	2.3	0.28(−0.21-0.66)	8	0.3
		-OPC	17	0.15	−0.5	0.64(0.24-0.86)	41	0.005[a]
		-SRT	13	0.10	1.8	0.53 (−0.02-0.84)	29	0.06
Western Europe	All	----	22	0.03	4.0	0.19 (−0.25-0.57)	4	0.4
		-OPC	21	0.05	3.0	0.40 (−0.04-0.71)	16	0.07
		-SRT	17	0.05	3.5	0.42 (−0.07-0.75)	18	0.09
	Solid tumors	---	20	0.04	3.7	0.22 (−0.24-0.61)	5	0.3
		-OPC	19	0.07	2.4	0.49 (0.04-0.77)	24	0.03[a]
		-SRT	15	0.06	2.8	0.53(0.02-0.82)	28	0.04[a]
United States	All (IARC)[b]	-SRT	13	0.02	3.9	0.34 (−0.26-0.75)	11	0.3
	(SEER)	---	20	0.02	4.2	0.20(−0.27-0.59)	4	0.4
	(SEER)	-SRT	16	0.05	3.6	0.45 (−0.06-0.77)	20	0.08
	Solid tumors (IARC)[c]	-SRT	11	0.03	3.5	0.42 (−0.24-0.82)	17	0.2
	(SEER)	----	17	0.03	3.9	0.22 (−0.29-0.64)	5	0.4
	(SEER)	-SRT	13	0.07	2.8	0.55 (0.01-0.85)	30	0.05[a]

[a]Statistically significant $P \leq 0.05$; [b]17 sites including SRT; [c]15 sites including SRT

No significant associations ($P \geq 0.1$ in all cases) were found for *TP53* mutation rates and their respective cancer exponents for 5 major topographic cancer sites (n_i: brain, breast, colorectum, liver, and lung) in different countries (**Table 3**). Similarly, no association ($P \geq 0.2$ in all sites) was found between the *TP53* mutations and the cancer exponent for the principal morphologic tumors of the 5 sites.

The variation in the international *TP53* mutation rates for brain, breast, colorectum, liver, and lung is small, with SD values of 31, 22, 20, 51, and 40% of the mean, respectively: liver has the largest percentage *TP53* mutation rate variation (mean 24 ± 12 SD) between countries. Except for the breast cancer exponent, the respective cancer exponents generally have similar or even smaller variation than the mutation rates, with SD values for brain, breast, colorectum, liver, and lung of 18, 55, 10, 24, and 16% of the mean, respectively. The variation of *TP53* and cancer exponents among countries of the 5 principal morphologic tumors is of the same order as that of the corresponding topographic sites.

Lastly, prostate cancer, with only 5 countries having ≥50 *TP53* samples, yielded a non-significant association (R^2 = 2%, P = 0.8) between percentage *TP53* mutation rates, mean 13 ± 10 SD, and cancer exponents, mean 11.7 ± 1.7 SD.

Relationship between body mass index and cancer incidence by country

A non-*TP53* influence was explored for brain, breast, colorectum, liver, and lung topographic cancer sites and their principal morphologic tumors. Only linear regression analyses of breast cancer and breast carcinoma yielded significant associations (R^2 = 42%, P = 0.001, cancer and R^2 = 52%, P = 0.003, carcinoma) between age-standardized body mass index[20] and cancer exponents in 22 and 15 countries, respectively (**Table 3**). Limited to 5 countries with adequate *TP53* data, or extended to 24 countries (see "Materials and Methods"), prostate cancer, unlike breast cancer, gave a non-significant association with body mass index (n = 5, R^2 = 23%, P = 0.4; n = 24, R^2 = <0.1%, P = 0.9).

Discussion

Databases that aggregate data from national or international sources, such as the IARC and SEER databases used in this study,

have intrinsic limitations due to different resources, methods of interpretation, and analysis.[21] Notwithstanding these limitations and the analytical methods employed in this study, the linear regression analyses led to quantification of significant relationships between *TP53* mutation rates and the incidence cancer exponent.

To assess a potential causal role for *TP53* mutations in the aging-related rise in cancer rates, I have used the findings of this study and previous studies to evaluate if the 9 criteria for causation established by epidemiologist Bradford-Hill are satisfied[22,23] (**Table 4**). The significant associations found address the "strength of association" criterion. In addition, the "consistency" Bradford-Hill criterion is met by the fact that the slope and intercept values of the *TP53* mutation rates and the cancer exponents are unfailingly positive and of the same magnitude regardless of the geographical area or the cancer categories analyzed in this study. While large uncertainties remain, this study shows that worldwide (**Table 2**), about a quarter of the aging-related exponential rise in the diagnosed tumors at all sites, excluding OPC, could be assigned to *TP53* mutations. *TP53* mutations have a smaller involvement in Western Europe and the United States, and a larger involvement in Japan (accounting for approximately one-third of the aging-related rise), perhaps because of other factors such as the low rate of obesity.

Unlike the 3 other regions analyzed solely on the basis of IARC databases, the United States data for *TP53* mutation rates and cancer exponents did not yield a significant relationship either for incidence of all cancer sites or solid tumors, even when SRT were excluded (**Table 2**). Of the 4 regions, United States data in the IARC *TP53* database included the lowest number of IARC GLOBOCAN cancer sites, adequately represented by 50 or more *TP53* mutation samples (all cancer sites, n = 17). However, I obtained a weakly significant association for solid cancers excluding SRT by alternatively employing SEER rather than IARC cancer data for the United States, increasing the number of all cancer sites to 20, supporting the importance of including a high number of cancer sites in this type of analysis.

Despite the associations found between the incidence cancer exponents K of various cancer sites n and the *TP53* mutation rates in specific geographical regions r_i, i.e., $TP53(n)r_i$ vs. $K(n)r_i$, similar significant relationships were absent between the *TP53* mutation rates in various countries N and the cancer exponents of specific cancer sites n_i, i.e., $K(N)n_i$ vs. $TP53(N)n_i$, or their principal tumors, i.e., $TP53(N)T_i$ vs. $K(N)T_i$. One possible reason for this discrepancy is the larger range of *TP53* mutation rates among cancer sites globally, varying ~7-fold from a low value of 6% for cervix uteri to a high value of 47% for ovarian cancers (**Table 1**). However, the range is narrower for specific cancer

Table 3. Regression analysis by country of cancer exponents from incidence ASR for 5 major topographic cancer sites (and prostate cancer) and their corresponding principal morphologic sites, vs. *TP53* mutation rates or body mass index (BMI) for individuals aged 40–44 y to 65–69 y (both sexes, except for breast and prostate)

Cancer site	Countries N	TP53 mutations or BMI	TP53 mutations, mean ± SD %	Cancer exponents, mean ± SD	Slope	R (95% CI)	R² %	P
Brain	11	*TP53*	27 ± 8	3.0 ± 0.5	0.00	0.0003(−0.60-0.60)	<0.1	0.99
	11	BMI			0.14	0.48(−0.17-0.84)	23	0.1
Brain, glioblastoma NOS	8	*TP53*	29 ± 6	2.9 ± 0.5	−0.01	0.08(−0.66-0.74)	0.7	0.8
Breast	22	*TP53*	23 ± 5	1.9 ± 1.0	−0.02	0.10(−0.33-0.50)	1	0.7
	22	BMI			0.38	0.65 (0.31-0.84)	42	0.001[a]
Breast, carcinoma NOS	15	*TP53*	22 ± 4	1.9 ± 0.9	0.00	0.006(−0.51-0.52)	< 0.1	0.98[a]
	15	BMI			0.37	0.72 (0.33-0.90)	52	0.003[a]
Colorectum	19	*TP53*	43 ± 9	5.7 ± 0.5	0.02	0.37(−0.10-0.71)	14	0.1
	19	BMI			0.09	0.27(−0.21-0.65)	8	0.3
Colorectum, adenocarcinoma NOS	11	*TP53*	49 ± 10	5.7 ± 0.5	0.02	0.44(−0.22-0.82)	19	0.2
Liver	14	*TP53*	24 ± 12	5.0 ± 1.2	0.01	0.13(−0.43-0.62)	2	0.7
	14	BMI			0.04	0.069(−0.48-0.58)	0.4	0.8
Liver, hepatocellular carcinoma NOS	13	*TP53*	24 ± 13	5.1 ± 1.2	−0.02	0.10(-0.48-0.62)	1	0.7
Lung	18	*TP53*	38 ± 15	6.4 ± 1.0	0.01	0.15(−0.34-0.58)	2	0.5
	18	BMI			0.09	0.15(−0.34-0.58)	2	0.6
Lung, non-small cell carcinoma	12	*TP53*	34 ± 13	6.2 ± 1.1	0.04	0.41(−0.21-0.80)	17	0.2
Prostate cancer	5	*TP53*	13 ± 10	11.7 ± 1.7	−0.03	0.15(−0.84-0.91)	2	0.8
	5	BMI			−0.44	0.48(−0.70-0.96)	23	0.4
	24	BMI			0.02	0.021(−0.39-0.42)	<0.1	0.92

[a]Statistically significant.

Table 4. *TP53* mutations and the aging-related rise in cancer incidence: Bradford-Hill criteria assessment[22]

Criterion	Summary (based on Höfler[23])	*TP53* mutations and the rise in adult cancer
Strength of association	The stronger the association, the more likely a causal component	*TP53* is the most frequently mutated gene in cancer. Mutations are found in 6–47% of tumors from 25 important cancer sites (**Table 1**), but mutation rates can be much higher for specific cancers.[21]
Consistency	A relationship is observed repeatedly	More studies have found an association between cancer and *TP53* mutations than any other genetic mutation[1,18]
Specificity	A factor influences specifically a particular outcome or population	*TP53* mutations elevate cancer susceptibility of many tumors, and are also a inherited characteristic of the Li-Fraumeni syndrome[24]
Temporality	The factor must precede the outcome it is assumed to affect	Preneoplastic *TP53* mutations confer a growth advantage in mouse mammary tumors[25] and human esophageal cancers,[26] but evidence is less convincing for human gastric tumors[27]
Biological gradient (dose–response relationship)	The outcome increases monotonically with increasing exposure to the factor	**Figure 2A** shows that the cancer exponent for all cancers, evaluated from worldwide incidence rates, increases with the presence of *TP53* mutations. In Li-Fraumeni syndrome, age of onset of cancer is directly related to the *TP53* transactivation functionality[28]
Biological plausibility	The observed association can be plausibly explained by biological explanations	*TP53* mutations increase glycolysis[29] and reduce apoptosis of neoplastic cells, and are also associated with genomic instability leading to cancer in mice and humans[30-32]
Biological coherence	A causal association should not fundamentally contradict present substantive knowledge	There are only a few exceptions[11,12] to the general finding that inactivating *TP53* mutations are associated with cancer. Results given here show 2.5-fold higher *TP53* mutation rate in epithelial tumor sites than in less-common, non-epithelial cancers.
Experimental evidence (including reversibility)	Cause is best shown by randomized experiments (or removal of cause leads to reduced effect)	In vivo evidence supports a causal role for *TP53* deficiencies in decreasing cancer latency and increasing susceptibility.[33,34] Reversibility has been demonstrated in two recent studies.[9,10] However, there is an absence of randomized experiments involving preneoplastic *TP53* mutations.
Analogy	An effect has already been shown for analogous factors, exposures, and outcomes	Overexpression of anti-apoptotic proteins (e.g., Bcl2, Mdm2)[35] and genes that induce genomic instability (e.g., *MYC* and *RAS*)[36,37] increase susceptibility to many cancers. Other gene deficiencies that, like mutated *TP53*, disable p53 function (e.g., *PTEN*) also increase cancer risk.[19]

sites; for example, the *TP53* mutation rate for breast cancer varies between 7% for Greece and 29% for Italy.

A second possible reason for the lack of association is that additional factors, other than differing *TP53* mutations by country, may have a strong influence on their cancer exponents. For example, for breast cancer, the cancer exponents of 5 Asian countries (China, Chinese Taiwan, India, Japan, and Korea) were statistically much smaller (~10-fold) than the values from the 17 non-Asian countries (means 0.2 ± 0.8 SD, 2.4 ± 0.4 SD, respectively; *t* test, *P* = 0.003). However, the corresponding *TP53* mutation rates are similar for these Asian and non-Asian countries (means 23 ± 7% SD, 23 ± 5% SD, respectively; *t* test, *P* = 0.999). An exploratory analysis of the relation between the body mass index and breast cancer exponents in various countries yielded a highly significant association, raising the possibility that obesity has a greater influence than *TP53* mutations for breast and perhaps other cancers.[16,17,38]

There are good grounds for Bradford-Hill "specificity" criterion (**Table 4**), as cancer susceptibility is an inherited property of Li-Fraumeni syndrome (characterized by germ line mutation of one *TP53* allele),[24] in which the age of onset of cancer is directly related to the *TP53* transactivation functionality.[28] Conversely, above-normal levels of p53 likely play a role in reducing cancer

incidence but also lifespan in those affected by Huntington disease.[39]

A stronger relationship between all cancer *TP53* mutation rates and cancer exponents occurs when excluding prostate cancer or solid cancers, the latter mainly due to the removal of multiple myeloma. These two cancers commonly have analogous factors to *TP53* mutations that reduce p53 protein activity. In the case of prostate cancer, up to 70% of tumors are devoid of a *PTEN* allele that regulates p53 stability.[40] In addition to the *TP53* mutational status of multiple myeloma being controversial, its *TP53/Mdm2* signaling pathways can be impaired by epigenetic silencing of p53-inducible microRNAs.[41]

No significant associations between *TP53* mutation rates and mortality cancer exponents were found. Yet previous studies have found an effect of *TP53* mutations on both cancer prognosis and outcomes, influencing not only tumor initiation, but also metabolism, angiogenesis, immunology, and invasion.[28,29,42] In **Table 4**, I note studies that support the Bradford-Hill criterion of "temporality", in which *TP53* mutations precede cancer incidence.[25,26] However, other studies, for example involving adenoma, a colonic polyp that is a precursor of colorectal cancer, indicate ambiguity as to when in tumor development a mutation in the *TP53* gene is acquired and the tumor transforms into an

invasive carcinoma.[43] For acute lymphoblastic leukemia patients, *TP53* mutations are about 10-fold more common at relapse than at initial presentation, possibly because *TP53* mutations occur during the course of treatment.[44] Nevertheless, in acute myeloid leukemia patients, a low *TP53* mutation rate is found both at presentation and relapse. Therefore, in this leukemia *TP53* mutations more likely occur before diagnosis.

Many factors have been proffered to explain the steep increase in the incidence of cancer with aging. Possible factors not specifically related to *TP53* include obesity, viruses, cumulative exposure to carcinogens such as radiation, immunological decline, less effective DNA repair; exhaustion of stem cells, and accumulation of senescent cells, pre-existing lesions, and somatic mutations.[45,46] Factors that can be related to p53-gene mutations include imbalance between cell birth and death, chromosomal instability and gene amplification.[47] In addition, Feng et al.[48] have identified a *TP53* factor in which mutation changes may only partially represent the whole *TP53* effect with aging. They found the *TP53* transcriptional activity and p53-dependent apoptosis response become less efficient in old age by exposing mice to gamma-radiation (5 Gy). The declining efficiency of p53-mediated apoptosis, senescence, or repair in retort to stress is one plausible explanation among others behind the general rise in chromosomal defects, somatic DNA mutations, and tumor incidence in older individuals.[45] A cascade of mutations may arise not only from the functional loss of a gatekeeper gene, such as *TP53* and other *TP53* pathway mutations (*BCL2*, *MDM2*, *MYC*, and *RAS*),[35-37] but by alternative means, such as telomere loss, defective DNA repair (i.e., caretaker gene mutations), and DNA-damaging agents such as radiation or viruses. Therefore, the high prevalence of the *TP53* mutations among the multiple mutations of cancers, together with the almost universal occurrence of telomerase activation, implies that *TP53* mutations may be linked to the neoplastic process, which preferentially utilizes glycolytic pathways[29] and bypasses *TP53*-induced senescence[4] to achieve immortality.

Somatic mutations in different tumor types can range from a few to more than a thousand,[49,50] although most are harmless passenger mutations, not driver mutations and therefore not cancer genes.[51] The number of driver mutations in general (rather than *TP53* in particular) may be associated with the cancer exponent of incident cancer rates. For example, testicular cancer and common leukemias (e.g., acute lymphoblastic leukemia and acute myeloid leukemia) are associated with few somatic mutations,[49] infrequent presence of *TP53* mutations (≤11%),[44] and cancer exponents with a relatively low value (**Table 1**). Conversely, epithelial cancer sites, which are dominant in adult humans, have on average a 2.5-fold higher rate of *TP53* mutations compared with non-epithelial cancer sites, providing a possible explanation for the preferential promotion of epithelial cancers.

Genomic instability is particularly effective at advancing the multistage process of inducing epithelial cancers, as shown in humans and animal studies.[47,52] Genomic instability is also a hallmark of cancer cells generally, manifested by chromosomal mutations and aneuploidy, the latter being the most common chromosomal defect associated with cancer.[30-32] An important question is whether *TP53* mutations are an important cause or result of genetic instability.[27,53] Indirect support for a causal role arises from the fact that childhood cancers have a lower rate of *TP53* mutations, fewer somatic mutations, and less evidence of genomic instability than adult cancers.[54,55] An experiment in mice by Schmitt et al.[32] supports a causal role of *TP53* more directly, finding that *TP53* loss sets off a chain reaction of genetic instability and aneuploidy, leading to lymphoma.

Hence, this assessment of the association between the candidate causative agent, *TP53* mutations, and the aging-related rise in cancer finds grounds for a causal role based on my results and previously reported literature, meeting all 9 Bradford-Hill criteria (**Table 4**).[22] This assessment was performed in a manner similar to Gazdar et al.,[56] who showed that 8 of the 9 Bradford-Hill criteria were addressed for the universally accepted relationship between the human papillomavirus and cervical cancer. Of these 8 criteria, the "strength of association" for this virus is very persuasive, present in more than 90% of cervical tumors. This is higher than the percentage of *TP53* mutations found in cancers in general, yet similar to *TP53* mutations in particular tumors, e.g., 70% in small cell lung cancers.[21] For human papillomavirus and cervical cancer, the "biological gradient" is unclear, whereas significant associations have been recorded (**Table 2**) for the presence of *TP53* mutations and the rise in adult cancer incidence. Although *TP53* mutations and consequent *TP53* inactivation satisfy all 9 Bradford-Hill criteria for causality, there is still reasonable doubt about *TP53*'s role in the aging-related rise in cancer. Other events, such as genetic instability and unidentified aspects of the aging process, may be complicit or indeed more important than *TP53*.

In summary, numerous publications have linked *TP53* mutational inactivation with cancer, yet an overall quantification of the influence of this genetic mutation on the general rise in cancer incidence with aging has not previously been reported. In this work, significant relationships were identified between somatic *TP53* mutation rates and the incidence cancer exponent, regardless of the geographical area analyzed; albeit, the association is relatively weak in the United States. This supports a role for elevated levels of this inactivated gene in the rise of cancer incidence with aging, independent of sex, which is probably linked to a tumor's requirement for genomic instability to escape replicative senescence and to utilize glycolytic pathways. The current relationship found between *TP53* mutations and cancer incidence does not explain the declining cancer rates in the very elderly: one possibility is that the p53 gene exhibits antagonistic pleiotropy, suppressing cancer at the cost of accelerated aging.[57,58] *TP53* mutations probably account for about one-quarter of the aging-related increase in all cancers worldwide, and an even larger proportion of the aging-related increase in Japan, where obesity is uncommon. Therefore, productive avenues of future research include improving the consistency and decreasing the uncertainty of the *TP53*/cancer data analyzed, and determining by multivariant analysis whether a major part of the steep rise in cancer incidence with aging involves obesity or deficiencies in one of the many genes that are part of the *TP53* signaling pathway.

Materials and Methods

TP53 mutations in cancer incidence and mortality data
Three large data sets were employed to provide epidemiologic data on cancer incidence or mortality, and *TP53* mutations. The IARC GLOBOCAN Cancer Database[59] provides ASR data (2008) in 5 y age groups for adults, covering 27 cancer sites in total for males, females, and both sexes. For United States incidence ASR data, I used SEER cancer statistics[60] in addition to the GLOBOCAN database. The percentage of cancer samples with *TP53* mutations for the cancers studied was obtained from the IARC *TP53* Mutation Database, R15 version, November 2010.[3,61]

The prevalence of *TP53* mutations worldwide for the incidence of all cancers ($n = 25$) and for solid tumors ($n = 23$) was calculated for both sexes as the sum of the product of different cancers' *TP53* mutation rates and ASR, divided by the total ASR.

Regional *TP53* mutations and cancer incidence analyses
Analyses were performed for four geographical regions: worldwide, Japan, Western Europe, and the United States. The latter three regions were chosen because of the availability of *TP53* mutation data (15 000–20 000 tumors sampled). Tumors were identified in the *TP53* database (**Table 1**) mainly by their anatomical site (topography), but also by their histology (morphology).

The cancer exponent for each of the cancers was calculated from the linear regression analysis of the log-transformed incidence or mortality ASR as the dependent variable and the log-transformed median age of 6 consecutive 5 y age groups as the independent variable. A small allowance of 5 y was made for lag between incidence and mortality ASRs. Accordingly, the cancer exponents were determined from 40–44 to 65–69 y of age for incidence ASR and from 45–49 to 70–74 y of age for mortality ASR.

Additional linear regression analyses were then conducted by region r_i, i.e., r_1–r_4, for each of the cancer sites n with the percentage of samples with *TP53* mutations as the independent variable and the cancer exponent as the dependent variable, i.e., $TP53(n)r_i$ vs. $K(n)r_i$. Although there are large differences in age-dependent ASR (**Fig. 1A**) and in lifetime accumulated cancer rates for males and females, I did not account for sex differences in *TP53* mutations. There is little evidence of sex differences in *TP53* mutations human cancers, although there are exceptions, such as a higher rate of *TP53* mutations in lung cancer among female smokers than among males.[21]

The same analysis was conducted for all cancers and for solid tumors. Cancer sites for which there are less than 50 tumors were analyzed for *TP53* mutations in the IARC *TP53* Mutation Database were excluded, on the recommendation of the database manager. For each geographical region examined, the number of cancer sites with adequate samples varied, making the included sites for all cancers and for solid tumors variable by region. For all cancers, the highest number of sites was 25 (*n*). Analyses were first conducted for males and females for all cancers and followed by both sexes for all cancer and solid tumors, including OPC or SRT and excluding OPC or SRT. Prostate cancer, which had a regression standardized residual of ≥3.0, was identified as an outlier.

In addition, the slopes and means of the analysis of *TP53* mutations vs. cancer exponents for each pair of regions (e.g., Japan:Western Europe) were compared by ANCOVA.

TP53 mutation rates and cancer incidence data by country
Finally, I examined for each country *N* whether the cancer exponent value $K(N)n_i$ for a specific cancer n_i was influenced by the country *TP53* mutation rate, i.e., $TP53(N)n_i$. This relationship was assessed for 5 major cancer sites n_1–n_5: brain, breast—females only, colorectum, liver, and lung) for which the IARC *TP53* Mutation Database has worldwide data from >5000 tumors for each cancer site. Prostate cancer was also assessed, although it is represented by only 1400 samples in the IARC *TP53* Mutation Database accessing the R16 version. Countries with 50 or more tumor samples in the *TP53* database for the cancer site were included.

As well, the relationship between *TP53* mutations and cancer exponent, i.e., $TP53(N)T_i$ vs. $K(N)T_i$ was studied for the principal tumor T_i of the 5 sites (T_1–T_5: glioblastoma NOS, carcinoma NOS—females only, adenocarcinoma NOS, hepatocellular carcinoma NOS, and non-small cell carcinoma, respectively). IARC *TP53* data for cancer sites was initially found by topography, whereas the *TP53* data on principal tumors was found by their morphology.[3,61]

Body mass index and cancer incidence data by country
A non-*TP53* influence, obesity, was briefly studied by use of age-standarized, mean body mass index (kg m^{-2}) data by country provided by Imperial College London, School of Public Health[20] for male and female adults in the year 2008. For prostate cancer, the number of countries was arbitrarily extended from 5 countries (with adequate *TP53* data) to 24 countries by adding countries with adequate breast cancer *TP53* data.

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