Selenium in cancer prevention: a review of the evidence and mechanism of action

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Se is an unusual trace element in having its own codon in mRNA that specifies its insertion into selenoproteins as selenocysteine (SeCys), by means of a mechanism requiring a large SeCys-insertion complex. This exacting insertion machinery for selenoprotein production has implications for the Se requirements for cancer prevention. If Se may protect against cancer, an adequate intake of Se is desirable. However, the level of intake in Europe and some parts of the world is not adequate for full expression of protective selenoproteins. The evidence for Se as a cancer preventive agent includes that from geographic, animal, prospective and intervention studies. Newly-published prospective studies on oesophageal, gastric-cardia and lung cancer have reinforced previous evidence, which is particularly strong for prostate cancer. Interventions with Se have shown benefit in reducing the risk of cancer incidence and mortality in all cancers combined, and specifically in liver, prostate, colo-rectal and lung cancers. The effect seems to be strongest in those individuals with the lowest Se status. As the level of Se that appears to be required for optimal effect is higher than that previously understood to be required to maximise the activity of selenoenzymes, the question has been raised as to whether selenoproteins are involved in the anti-cancer process. However, recent evidence showing an association between Se, reduction of DNA damage and oxidative stress together with data showing an effect of selenoprotein genotype on cancer risk implies that selenoproteins are indeed implicated. The likelihood of simultaneous and consecutive effects at different cancer stages still allows an important role for anti-cancer Se metabolites such as methyl selenol formed from γ-glutamyl-selenomethyl-SeCys and selenomethyl-SeCys, components identified in certain plants and Se-enriched yeast that have anti-cancer effects. There is some evidence that Se may affect not only cancer risk but also progression and metastasis. Current primary and secondary prevention trials of Se are underway in the USA, including the Selenium and Vitamin E Cancer Prevention Trial (SELECT) relating to prostate cancer, although a large European trial is still desirable given the likelihood of a stronger effect in populations of lower Se status.

Selenium: Cancer: Mechanism: Selenomethyl-selenocysteine: Selenoprotein single-nucleotide polymorphism

Se is an essential trace element like no other. Its unique redox chemistry has been exploited by biological systems since the advent of dioxygen in the earth's environment created a requirement for a two-electron detoxification system for dealing with peroxides (Frausto da Silva & Williams, 2001). Its crucial role is underlined by the fact that it is the only trace element to be specified in the genetic code (RJP Williams, personal communication), as selenocysteine (SeCys; the 21st amino acid), which when incorporated into selenoproteins, protects tissues and membranes from oxidative stress and controls cell redox status (Rayman, 2000). As will be seen later, SeCys is 'dramatically different from the other twenty amino acids in the mode of its incorporation and basic biosynthetic steps' (Hatfield & Gladyshev, 2002) and this complex insertion machinery for selenoprotein production has implications for the Se requirements for cancer prevention.

Table 1. Some selenoproteins of particular relevance to cancer

Selenoprotein	Function	References
Glutathione peroxidases (GPx; particularly GPx1, cytosolic; GPx2, gastrointestinal; GPx4, phospholipid)	Antioxidant enzymes: remove H_2O_2, lipid and phospholipid hydroperoxides thereby maintaining membrane integrity, modulating eicosanoid synthesis, modifying inflammation and the likelihood of propagation of further oxidative damage to biomolecules	Spallholz et al. (1990), Diplock (1994), Sunde (1997), Allan et al. (1999)
15 kDa selenoprotein	Associated with the endoplasmic reticulum: may be involved in the regulation of protein folding	Korotkov et al. (2001)
	Gene located in a region often altered in human cancers	Hu et al. (2001)
	Expressed at high levels in normal liver and prostate but at reduced levels in the corresponding malignant organs; may protect prostate cells against development of carcinoma	Behne et al. (1997)
Selenoprotein P	Found in plasma and associated with endothelial cells. Antioxidant and transport functions	Burk et al. (2003)
	Scavenger of peroxynitrite, particularly at the endothelium	Arteel et al. (1999)
	Is down regulated in human tumours	Calvo et al. (2002)
Thioredoxin reductases (1, 2 and 3)	NADPH reduction of thioredoxin and other substrates; reduction of nucleotides in DNA synthesis; regeneration of antioxidant systems; maintenance of the intracellular redox state, critical for cell viability and proliferation; regulation of gene expression by redox control of binding of transcription factors to DNA	Allan et al. (1999)
	More highly expressed in cancer cells than in normal cells and its expression is repressed by p53	Gladyshev et al. (1998)

Evidence is accruing, some of which will be presented, that the level of intake of Se affects the risk of cancer and may even inhibit its spread from a primary tumour. Since UK deaths from cancer in adults now outnumber deaths from IHD and stroke, and approximately one in three of the European population will be diagnosed with cancer during their lifetime (CancerStats, 2004a,b), it is timely to consider the potential of Se for cancer reduction.

The nature of the Se species involved in anti-cancer processes is still a matter of speculation and much ongoing experimental work. Whether the selenoproteins are crucial to the anti-cancer effects requires some understanding of the biosynthetic machinery involved and of the function of some of the selenoproteins most likely to be relevant to cancer. These issues will be addressed.

Selenoproteins

Biosynthesis

Unlike the other twenty amino acids, SeCys is biosynthesised on its own tRNA, Sec tRNA[Ser]Sec, from selenophosphate as the Se source. Sec tRNA[Ser]Sec has many unusual features, including its long length (Hatfield & Gladyshev, 2002). The insertion of SeCys is specified by the UGA codon in mRNA. However, as UGA is also a stop codon the presence of a stem–loop structure in mRNA, a SeCys insertion sequence (SECIS) element, downstream from UGA in the $3'$-mRNA-untranslated region is also required for UGA to be read as SeCys. SECIS elements function by recruiting additional factors, including the SECIS-binding protein, the SeCys-specific elongation factor and Sec tRNA[Ser]Sec, to form the large SeCys insertion complex required for the synthesis of selenoproteins and known as the selenosome (Berry et al. 1991, 1993; Hatfield &

Gladyshev, 2002). The human selenoproteome consists of twenty-five selenoproteins (Kryukov et al. 2003).

Some selenoproteins of particular relevance to cancer

The functions of many of the twenty-five human selenoproteins are as yet unknown, although they generally participate in antioxidant and anabolic processes (Hatfield & Gladyshev, 2002). Selenoproteins that may be relevant to cancer risk are described in Table 1 and include a number from the glutathione peroxidase (GPx) family, the 15 kDa selenoprotein (Sep15), selenoprotein P and the thioredoxin reductases, although a beneficial role of the thioredoxin reductases in cancer prevention is doubtful.

Selenium intakes and status of adults in different countries

If Se may protect against cancer, an adequate intake of Se is desirable. Whether the intake of Se is adequate is, however, questionable in much of Europe and some other parts of the world. Mean intake levels in a number of countries (Combs, 2001; Rayman, 2004) are shown in Fig. 1, which also indicates the range of Se intake believed to be required for optimal activity of plasma GPx (Thomson et al. 1993; Duffield et al. 1999). It is clear that the level of intake in Europe and some parts of China is not adequate for full expression of GPx. (According to Combs (2001), the same may be true of other parts of the world, as there is little or no information on Se intake or status for most of Africa, South America and central and south Asia.) Furthermore, an updated study of Se requirements by Burk's group in collaboration with Chinese colleagues (Xia et al. 2005) has shown that full expression of selenoprotein P requires a greater Se intake than that required
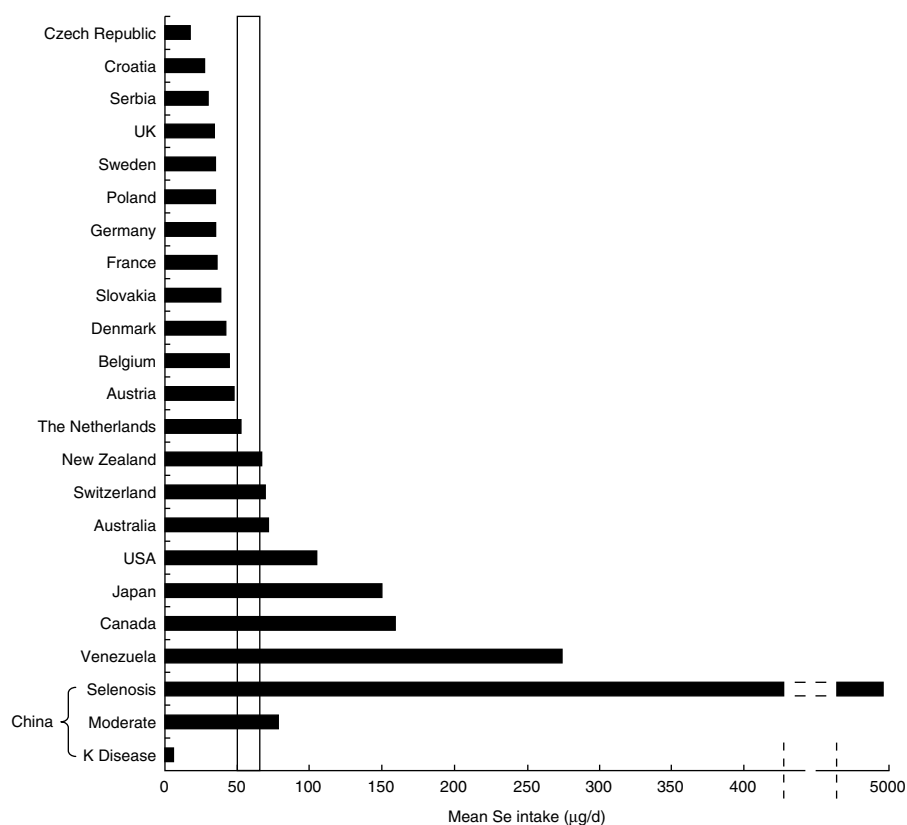
Fig. 1. Mean selenium intake levels (μg/d; ■) in different countries (Combs, 2001; Rayman 2004) and the range of selenium intake believed to be required for optimal activity of plasma glutathione peroxidase (Thomson *et al.* 1993, Duffield *et al.* 1999; □).

for full expression of plasma GPx. Thus, it is even more likely that current intakes are inadequate for optimising the protective effects of the selenoproteins. Indeed, there is evidence that will be outlined that suggests that levels of Se intake that are supra-nutritional may be required to reduce cancer risk (Combs, 2001; Rayman, 2002).

Evidence for an effect of selenium on cancer risk

The evidence for Se as a cancer preventive agent has been reviewed ably by a number of researchers (for example, see Combs & Grey, 1998; Ip, 1998; Combs & Lü, 2001; Knekt, 2002; Whanger, 2004; Combs, 2005), and includes findings from *in vitro*, animal, geographic (ecological) and prospective studies, and from interventions with Se. Such evidence will be summarised and updated, although *in vitro* studies and studies on Se compounds that cannot arise from food sources will only be referred to briefly (for more detail, see Combs & Grey, 1998; Ip, 1998; Combs & Lü, 2001; Knekt, 2002; Whanger, 2004; Combs, 2005). Case–control studies will be excluded as it is not possible to distinguish between Se concentration as an indicator of cancer risk and Se concentration that is a consequence of the disease process (Overvad, 1998).

Animal studies

Extensive experimental evidence indicates that Se supplementation reduces the incidence of cancer in animals (Medina & Morrison, 1988; Combs & Gray, 1998; Combs & Lü, 2001). However, it is difficult to generalise from such studies and extrapolate to the human situation, as animal studies have generally used doses at least ten times greater than those required to prevent clinical signs of deficiency, which, on a per unit body-weight basis, are considerably higher than most human Se intakes. However, it is worth describing a supplementation study on male beagle dogs, a species that develops spontaneous prostate cancer, as the lower dose given is reasonable for man. Supplementation of the diet of sexually-intact elderly male dogs with Se, as selenomethionine or high-Se yeast, at 3 or 6 μg/kg body weight per d for 7 months was found to reduce DNA damage and up-regulate epithelial cell apoptosis in their prostates, while no such effects were seen in the dogs that were not supplemented (Waters *et al.* 2003). It appears that Se sensitises prostate epithelial cells so that cells with extensive DNA damage undergo apoptosis *in vivo*.

Geographical (ecological) studies

Since as early as the 1960s geographical studies have shown a consistent trend for populations with low Se intakes to have higher cancer mortality rates (Shamberger & Frost, 1969; Schrauzer *et al.* 1977; Clark *et al.* 1991). In one such study (Schrauzer *et al.* 1977), inverse correlations were observed between apparent dietary Se intakes estimated from food-consumption data in twenty-seven

countries and age-corrected mortality for a number of cancers, including that of the prostate. However, the value of evidence from this type of study is not rated very highly by epidemiologists.

Prospective and nested case–control studies

Knekt (2002) has tabulated the results of prospective studies of Se and cancer published up to the end of 1998. The following categories are included: all cancers; lung cancer; colo-rectal, gastrointestinal and stomach cancers; prostate cancer; female cancers; miscellaneous cancers that include cancers of the liver, bladder, mouth, pharynx, oesophagus and malignant melanoma. Of approximately seventy-two table entries, fifty entries show a lower risk associated with higher Se intake or status, although only in eighteen studies (25%) that included all cancers and cancers of the bladder, lung, ovary, prostate, stomach and thyroid is the risk significantly reduced.

More recent evidence that Se status can influence mortality from all cancers combined has been found in a cohort of 1389 male and female volunteers recruited in the Etude du Vieillissement Artériel (Akbaraly *et al.* 2005). Mean baseline plasma Se levels in the cohort were reported to be 86 μg/l, which is similar to levels in much of Europe. During the 9-year follow-up, 101 subjects died, fifty-five of them from cancer. The risk of mortality from cancer was shown to be increased fourfold in subjects in the bottom quartile of baseline plasma Se compared with those in the top quartile (relative risk (RR) 4·06 (95% CI 1·51, 10·92); $P = 0·006$).

The strongest evidence for a beneficial effect of Se from prospective studies appears to relate to lung cancer, oesophageal and gastric-cardia cancers and, most notably, prostate cancer. The risk of colo-rectal adenoma, a pre-cancerous condition, also seems to be affected.

Lung cancer. A recent meta-analysis of existing epidemiological evidence from sixteen studies has shown a significantly decreased risk of lung cancer (summary RR 0·74) associated with higher Se exposure (Zhuo *et al.* 2004; Table 3). The effects were found to occur primarily in populations of low Se exposure (defined as serum Se <100 μg/l or intake <55 μg/d). In studies carried out in high-Se areas (defined as serum Se >100 μg/l or intake >55 μg/d) protective effects appeared on moving from the lowest Se category to the second-lowest Se category, but increasing Se exposure thereafter appeared to have little further effect, suggesting the existence of a threshold effect.

Oesophageal cancer and gastric-cardia cancer. In a nested study from the Nutrition Intervention Trial in Linxian, China, significant inverse associations were found between baseline serum Se concentration as a continuous variable and death from oesophageal squamous cell carcinoma (RR 0·83 (95% CI 0·71, 0·98)) and gastric-cardia cancer [RR 0·75 (95% CI 0·59, 0·95)] in 1103 subjects randomly-selected from the larger trial cohort and followed for 15 years (Wei *et al.* 2004). When the subjects were classified by quartile of baseline Se, those in the highest quartile had a 65% significant reduction in the risk of death from oesophageal squamous cell carcinoma (RR 0·35

(95% CI 0·16, 0·81)) and a 69% significant reduction in the risk of death from gastric-cardia cancer (RR 0·31 (95% CI 0·11, 0·87)) when compared with the subjects in the lowest quartile. The mean population serum Se concentration in the cohort (73 μg/l) was relatively low. It has been suggested by Wei *et al.* (2004) that population-wide Se supplementation in regions of China with low serum Se levels and high rates of these cancers merits serious consideration.

Prostate cancer. Results from large prospective studies of prostate cancer (Knekt *et al.* 1990; Yoshizawa *et al.* 1998; Helzlsouer *et al.* 2000; Nomura *et al.* 2000; Brooks *et al.* 2001; Goodman *et al.* 2001; van den Brandt *et al.* 2003; Li *et al.* 2004) are shown in Table 2. Those published in 2003 and 2004 were large studies with 540 (van den Brandt *et al.* 2003) and 586 (Li *et al.* 2004) cases. Of the eight prospective studies listed seven show a reduced risk of prostate cancer overall for the highest *v.* lowest category of Se status, the risk being significantly reduced in five studies. When the analysis is confined to subjects who had advanced prostate cancer or a baseline prostate-specific antigen of >4 ng/ml, six of the eight prospective studies show a significant reduction in prostate cancer in the subjects in the highest category of Se status.

Although the study of Knekt *et al.* (1990) in Finland showed no relationship between serum Se concentration and prostate cancer risk, Platz & Helzlsouer (2001) have noted that the participants had circulating levels almost three times lower than those reported in the other studies (approximately 50 μg/l *v.* 150 μg/l). Thus, it may be possible that the concentration of Se in this cohort was below the threshold at which Se can exert a protective effect on prostate cancer risk. This possibility is given credence by the study of Nomura *et al.* (2000), which has shown that there is a protective effect (odds ratio (OR) 0·5) mainly in subjects with serum Se concentrations >147 μg/l, with an OR of approximately 1 in lower quartiles of plasma Se.

In a number of these studies (Yoshizawa *et al.* 1998; Nomura *et al.* 2000; van den Brandt *et al.* 2003; Li *et al.* 2004) the protective effect of Se has been shown to be stronger for advanced prostate cancer, i.e. disease that has spread beyond the prostate, than for localised disease. Furthermore, when data from the Physicians' Health Study were analysed according to baseline prostate-specific antigen level, the protective effect was found to be significant for all prostate cancers (both localised and advanced disease) but only in those with baseline prostate specific antigen >4 ng/ml (Li *et al.* 2004), again suggesting a major effect of Se on prostate cancer progression rather than initiation.

Two studies have suggested that smoking modifies the effect of Se. The Netherlands Cohort Study has shown by far the strongest effect of Se in ex-smokers (van den Brandt *et al.* 2003), while the inverse association between Se and prostate cancer was found to be mainly present in current or past cigarette smokers in the study of Nomura *et al.* (2000).

Colo-rectal adenoma. Colo-rectal adenoma is closely associated with subsequent development of colo-rectal cancer (Weingarten *et al.* 2005). Jacobs *et al.* (2004) have

Table 2. Large prospective studies of prostate cancer or advanced prostate cancer using tissue indicators of exposure

Reference	Study population	No. of cases	Indicator of exposure	Comparison: high v. low	RR†	95% CI	P (for trend)
Knekt et al. (1990)	Finland, general population	51	Serum	Quintile	1·15	–	0·71
Yoshizawa et al. (1998)	USA, health professionals	181	Toenails	Quintile	0·35‡	0·16, 0·78*	0·03
Nomura et al. (2000)	USA, Hawaii, Japanese ancestry	249	Serum	Quartile	0·5	0·3, 0·9*	0·02
	Non smoker	87			0·8	0·4, 1·9	0·93
	Ex-smoker	86			0·5	0·2, 1·1	0·03
	Current smoker	76			0·2	0·1, 0·8	0·02
	Localised disease	120			0·8	0·4, 1·8	0·76
	Advanced disease	64			0·3‡	0·1, 0·8	0·01
Helzlsouer et al. (2000)	USA, Washington County	117	Toenails	Quintile	0·58	0·29, 1·18	0·27
					0·38§	0·17, 0·85*	0·12
Goodman et al. (2001)	USA, CARET		Serum	Quartile			
	Asbestos workers, current and ex-smokers	235			1·02	0·7, 1·6	0·69
	Retinol–β-carotene arm	111			0·75	0·41, 1·36	0·40
	Placebo arm	124			1·52	0·78, 2·79	0·12
Brooks et al. (2001)	USA, Baltimore	52	Plasma	Quartile	0·24	0·08, 0·77*	0·01
van den Brandt et al. (2003)	The Netherlands, Cohort Study	540	Toenails	Quintile	0·69	0·48, 0·99*	0·008
	Never smoker	72			1·19	0·48, 2·92	
	Ex-smoker	300			0·46	0·27, 0·79*	
	Current smoker	168			0·97	0·42, 2·22	
	Localised disease	189			0·72	0·42, 1·24	0·043
	Advanced disease	183			0·62‡	0·37, 1·05	0·020
Li et al. (2004)	USA, Physicians' Health Study	586	Plasma	Quintile	0·78	0·54, 1·13	0·16
	Baseline PSA >4 ng/ml	228			0·49	0·28, 0·86*	0·002
	Baseline PSA <4 ng/ml	293			0·77	0·48, 1·22	0·59
	Localised disease	348			0·97	0·64, 1·49	0·91
	Advanced disease	171			0·52‡	0·28, 0·98*	<0·05

CARET, β-Carotene and Retinol Efficacy Trial; RR, relative risk; PSA, prostate-specific antigen.
*The effect was significant.
†Highest category v. lowest category.
‡Advanced disease.
§Adjusted for BMI at age 21 years, education and interval (h) since last meal.

Table 3. Meta-analysis of existing epidemiological evidence from sixteen studies of selenium and lung cancer (Zhuo et al. 2004)

	RR*	95% CI
All subjects	0·74	0·57, 0·97
Low-Se areas	0·72	0·45, 1·16
High-Se areas	0·86	0·61, 1·22

RR, relative risk.
*High Se exposure v. low Se exposure.

carried out a pooled analysis of data from three studies that could be considered as prospective studies of Se and risk of colo-rectal adenoma. The Wheat Bran Fiber Trial (Alberts et al. 2000), the Polyp Prevention Trial (Schatzkin et al. 2000) and the Polyp Prevention Study (Greenberg et al. 1994) were 3–4-year interventions in subjects that had recently undergone adenoma removal, 1763 of whom had baseline serum or plasma Se levels measured. The risk of adenoma recurrence was not affected by any of the interventions. Analysis of pooled data showed that the subjects with baseline serum or plasma Se in the highest quartile (median 150 μg/l), when compared with those in the lowest quartile (median 113 μg/l), had a significantly lower risk of adenoma recurrence (OR 0·66 (95 % CI 0·50, 0·87). These results support previous findings that are suggestive of a beneficial effect of higher Se status on colo-rectal cancer risk (Jacobs et al. 2004).

Intervention studies including randomised controlled trials

Chinese trials. National Cancer Institute-sponsored trials in China for the prevention of oesophageal and gastric cancer have observed a reduction in total cancer mortality and a reduced incidence of oesophageal and gastric-cardia cancers in the intervention arm comprising Se, β-carotene and vitamin E (Blot et al. 1993; Mark et al. 2000). Although Se was not a single agent in these trials, it is likely to have been the most effective component, particularly in the light of subsequent studies (Wei et al. 2004). (As one of a number of agents in an Indian trial Se has also been shown to aid the remission of precancerous lesions of the oral cavity (Krishnaswamy et al. 1995; Prasad et al. 1995).)

Hepatocellular carcinoma is highly prevalent in China. In Qidong county, near Shanghai, its incidence is

Table 4. Nutritional Prevention of Cancer Trial (Clark *et al.* 1996, 1998): relative risk (RR) of cancer incidence and mortality in the selenium-treated group compared with the placebo group, by follow-up period (Duffield-Lillico *et al.* 2002)

Cancer		Follow-up until:	RR	95% CI	P
All sites	Mortality	31 December 1993	0·50	0·31, 0·80	0·002
		1 February 1996	0·59	0·39, 0·89	0·008
All sites	Incidence	31 December 1993	0·63	0·47, 0·85	0·001
		1 February 1996	0·75	0·58, 0·98	0·03
Lung	Incidence	31 December 1993	0·54	0·30, 0·98	0·04
		1 February 1996	0·70	0·40, 1·21	0·18
Colo-rectal	Incidence	31 December 1993	0·42	0·18, 0·95	0·03
		1 February 1996	0·46	0·19, 1·08	0·055
Prostate	Incidence	31 December 1993	0·37	0·18, 0·71	0·002
		1 February 1996	0·51	0·29, 0·87	0·009

particularly high. In this region approximately 15% of adults carry the hepatitis B surface antigen and these individuals are 200 times more likely to develop hepatocellular carcinoma. In a study in which 226 hepatitis B antigen carriers were randomised to a Se (200 μg)-enriched yeast tablet or a placebo, no case of hepatocellular carcinoma was reported to occur in the Se-supplemented group after 4 years, while seven subjects in the unsupplemented placebo group had developed hepatocellular carcinoma (Yu *et al.* 1997). However, as full details of the methodology of this study are not available, it is difficult to assess whether its protocol was sufficiently well-controlled or robust to be confident in its conclusions.

A recent systematic review and meta-analysis of antioxidant supplements for the prevention of gastrointestinal cancers has assessed the evidence for an effect of Se (Bjelakovic *et al.* 2004). Data from three Chinese trials were included, two of which used selenised yeast (Yu *et al.* 1997), while the third trial used Na_2SeO_3 (Li *et al.* 2000). Bjelakovic *et al.* (2004) concluded that, in contrast to other antioxidant nutrients, Se showed a significant beneficial effect, reducing the risk of hepatocellular carcinoma by 50% (RR 0·50 (95% CI 0·35, 0·71)).

The Nutritional Prevention of Cancer Trial and follow-up analyses. The strongest evidence of the efficacy of Se as an anti-cancer agent, particularly for prostate cancer, is provided by the Nutritional Prevention of Cancer (NPC) trial, carried out by Clark and co-workers (Clark *et al.* 1996, 1998; Duffield-Lillico *et al.* 2002, 2003*a,b*). Subjects (*n* 1312) with a history of non-melanoma skin cancer were randomised to placebo or 200 μg Se (as Se-enriched yeast)/d. After 4·5 years of treatment and 6·5 years of follow-up no effect was found on the primary end point of non-melanoma skin cancer. However, in those subjects receiving Se, significant secondary end-point effects of 50% lower total cancer mortality and 37% lower total cancer incidence were found, with fewer prostate, colo–rectal and lung cancers (Table 4). Follow-up analyses to the end of the blinded treatment period, a further 25 months, showed a reduced effect on total cancer, but while the protective effect on prostate cancer was maintained there was no longer a protective effect on lung and colo-rectal cancers (Duffield-Lillico *et al.* 2002; Table 4).

Although the initial finding that Se supplementation was not significantly associated with the incidence of basal-cell carcinoma (Cox proportional hazards model; hazard ratio (HR) 1·09 (95% CI 0·94, 1·26)) was confirmed in the follow-up analyses, the elevated risk of squamous-cell carcinoma and total non-melanoma skin cancer was raised by the extended period of treatment to significant levels (HR 1·25 (95% CI 1·03, 1·51) and 1·17 (95% CI 1·02, 1·34) respectively; Duffield-Lillico *et al.* 2003*b*). However, there are a number of reassuring factors that are relevant here: first, when a treatment lag of 2 years following randomisation was introduced, thus excluding lesions already in the course of development, the significant effect disappeared; second, when subjects were divided into tertiles according to baseline Se status, those in the bottom tertile (see earlier discussion), whose status resembled that found in Europe, did not have an increased risk of squamous-cell carcinoma (HR 0·87 (95% CI 0·62, 1·22)). Finally, it must be remembered that the subjects in the NPC trial were all patients with skin cancer whose skin had sustained heavy sun damage (Duffield-Lillico *et al.* 2003*b*).

The Nutritional Prevention of Cancer Trial subgroup analyses. The protective effect of Se was found to be confined to men, both in the initial and follow-up analyses, although the fact that there were many fewer women than men (319 *v.* 931) must be taken into consideration (Clark *et al.* 1996; Duffield-Lillico *et al.* 2002). As seen in some of the prospective studies discussed earlier, the protective effect of Se was found to be stronger in former smokers (Duffield-Lillico *et al.* 2002).

Analysis of treatment effect by initial plasma Se status in the NPC trial has shown that the strongest treatment effect was in subjects in the lowest tertile of plasma Se at baseline, i.e. those subjects whose plasma Se concentration was <106 μg/l at entry to the trial (Duffield-Lillico *et al.* 2002). Se supplementation was found to reduce total cancer incidence in this tertile by 49% (HR 0·51 (95% CI 0·32, 0·81)) (Duffield-Lillico *et al.* 2002) and prostate cancer incidence by 86% (HR 0·14 (95% CI 0·03, 0·61); Duffield-Lillico *et al.* 2003*a*) in the follow-up analyses. Most UK and European populations would fall into this tertile.

A significant interaction between baseline plasma Se and treatment was detected such that those subjects in the top tertile (>121·6 μg/l) that were supplemented with Se had a significantly increased risk of total cancer (HR 1·88 (95% CI 1·15, 3·05); *P* = 0·01; Duffield-Lillico *et al.* 2002).

Although this is a subgroup analysis of a secondary end-point analysis and must therefore be regarded with caution, it does raise queries about the advisability of supplementing individuals of already-adequate status (e.g. $\geq 120\,\mu g/l$) with Se.

Insights from the evidence presented

What lessons can be learned from the NPC trial? It would appear that plasma Se should reach approximately $120\,\mu g/l$ to optimise the anti-cancer effect of Se. This level is higher than that previously understood to be required to maximise the activity or concentration of selenoenzymes such as GPx (Thomson *et al*. 1993; Duffield *et al*. 1999), although ideas for optimum levels have recently had to be revised upwards as a result of new findings on requirements for selenoprotein P (Xia *et al*. 2005). Does this outcome mean that the selenoenzymes are not relevant to the anti-cancer effects of Se, or do some individuals have a higher Se requirement, perhaps as a result of single-nucleotide polymorphisms (SNPs) in their selenoprotein genes? This issue will be addressed as part of a general consideration of possible mechanisms by which Se may reduce cancer risk.

Selenium anti-cancer mechanisms

A number of mechanisms have been suggested to explain the anti-cancer effects of Se. These are summarised in Table 5. Although there is fairly general acceptance that methyl selenol (CH_3SeH) is involved in the anti-cancer effects of Se at supra-nutritional doses, as will be explained below, evidence is accruing, some from effects of functional selenoprotein polymorphisms, that the selenoenzymes do play a role, particularly at nutritional levels of intake. Se in selenoproteins can reduce oxidative stress and limit DNA damage, both of which have been linked to cancer risk. Some of these anti-cancer processes or pathways are discussed more fully later (p. 536).

Methyl selenol and its precursors

The *in vivo* production of small-molecular-weight Se metabolites such as CH_3SeH that have potent anti-cancer properties has been inferred from work carried out by a number of research groups (Ip, 1998; Jiang *et al*. 1999; Ip *et al*. 2000, 2002; Davis & Finley, 2003; Spallholz *et al*. 2004; Whanger, 2004). The metabolism of dietary forms of Se is shown in Fig. 2 (adapted from Combs, 2001; Rayman, 2004), from which it can be seen that CH_3SeH can be formed by the methylation of H_2Se as part of the Se excretory pathway. There is also some evidence that CH_3SeH can be formed directly from selenomethionine either by the action of a γ-lyase, also known as methioninase (Nakamuro *et al*. 1997; Wang *et al*. 2002; Spallholz *et al*. 2004) or by an α, γ-elimination reaction (Okuno *et al*. 2005). Alternatively, it can be formed from a storage form of Se, i.e. γ-glutamyl-selenomethyl (SeMe)-SeCys, that is present in plants of the Brassica and Allium families (Ip *et al*. 2000; Kotrebai *et al*. 2000; Whanger, 2004)

and probably accounts for the anti-tumour effects of Se-enriched broccoli and garlic (Ip *et al*. 2000; Davis & Finlay, 2003). Metabolism removes the γ-glutamyl group to give SeMe-SeCys, which is acted upon by a β-lyase to give CH_3SeH directly (Ip *et al*. 2000; Combs, 2001). There is a suggestion that the β-lyase is present at a higher level in cancer cells than in normal cells, ensuring greater exposure of the tumour cells to the anti-cancer agent (Spallholz *et al*. 2004).

Speciation studies have been carried out on Se-enriched yeast, the form of Se shown to be effective in most human interventions. These studies have shown the presence of small amounts of both γ-glutamyl-SeMe-SeCys and SeMe-SeCys, dependent on the method of extraction, inferring that CH_3SeH may be produced directly from the Se-enriched yeast without the necessity of conversion from selenomethionine, its major Se constituent (Goenaga Infante *et al*. 2004, 2005). As SeMe-SeCys has been found to be more than twice as effective as selenomethionine in reducing mammary tumours in rats (Whanger, 2004), even these small amounts may be important.

Precursors of CH_3SeH, typically methyl seleninic acid (CH_3SeO_2H) in experimental *in vitro* systems, have been shown to block progression of the cell cycle, induce apoptosis of cancer cells and inhibit the formation of new blood vessels, without which tumours cannot grow or metastasise (Ip, 1998; Jiang *et al*. 1999; Ip *et al*. 2000; Davis & Finley, 2003; Whanger, 2004). Processes by which these effects are achieved may involve redox cycling linked to oxidative stress-induced apoptosis, as described by Spallholz *et al*. (2004), and include changes in the expression of genes that control the cell-cycle checkpoint and regulate signalling pathways and caspase-mediated apoptosis (Dong *et al*. 2003). For instance, SeMe-SeCys activates caspase-3 in mouse mammary epithelial tumour cells *in vitro* (Unni *et al*. 2001) while CH_3SeO_2H is known to activate initiator caspases-1, 8, 10, and 12 (Zu & Ip, 2003). Apoptosis induced by CH_3SeO_2H in DU-145 and PC-3 human prostate cancer cells is principally initiated by caspase-8 and involves cell detachment as a prerequisite (Jiang *et al*. 2001; Zu & Ip, 2003). Caspase-12, an endoplasmic reticulum-resident caspase essential for endoplasmic reticulum stress-induced apoptosis, is also activated during apoptosis induced by CH_3SeO_2H in PC-3 cells, suggesting a possible role for endoplasmic reticulum stress in apoptosis induced by CH_3SeH (Zu & Ip, 2003).

Reduction of DNA damage

Evidence that Se can reduce DNA damage comes from studies in dogs and man. In a canine model of prostate cancer forty-nine elderly male beagle dogs, physiologically equivalent to 62–69-year-old men and similarly subject to prostate cancer, received nutritionally-adequate or supra-nutritional levels of dietary Se as selenomethionine or Se-enriched yeast for 7 months (Waters *et al*. 2005). DNA damage in the prostate was measured by the alkaline comet assay while Se was measured in toenails. The percentage of prostate cells with extensive DNA damage was found to fall with increased Se exposure up to a level of

Table 5. Some cellular processes and molecular pathways that may be involved in the anti-cancer effect of selenium

Anti-cancer processes or pathways	Selected evidence for Se involvement	Reference
Seleno-enzyme mechanisms Reduction of DNA damage	Se intake or status affects DNA damage in both human and animal studies	Karunasinghe et al. (2004), Kowalska et al. (2005), Waters et al. (2005); also, see p. 533
Reduction of oxidative stress	Levels of dietary antioxidant vitamins and carotenoids and SNP that affect antioxidant selenoproteins modify the effect of Se on cancer risk	See p. 535
Reduction of inflammation: inflammation promotes tumour growth (Caruso et al. 2004).	Selenoenzymes can reduce hydroperoxide intermediates in the cyclooxygenase and lipoxygenase pathways preventing the production of pro-inflammatory prostaglandins and leukotrienes	Rayman (2000)
Induction of phase II conjugating enzymes: detoxify carcinogens and reduce DNA adduct formation	Some selenocompounds e.g. methyl selenol (CH_3SeH), can up regulate phase II conjugating enzymes such as glutathione-S-transferase, increasing detoxification of carcinogens	Ip & Lisk (1997)
	Carcinogen adducts are reduced in liver and mammary gland of rats fed Se-enriched garlic, mushrooms and selenite	Davis & Finley (2003)
Enhancement of immune response: cytotoxic lymphocytes and natural-killer cells are able to destroy tumour cells	Se supplementation (Na_2SeO_3) enhanced the immune response of volunteers and cancer patients by increasing the numbers of cytotoxic lymphocytes and natural-killer cells	Kiremidjian-Schumacher et al. (1994, 2000)
Increase in tumour-suppressor protein p53: inhibits proliferation, stimulates DNA repair and promotes apoptotic death by acting as a transcription factor for several genes, including the damage-inducible gadd genes	SeMet can activate p53 through redox regulation of key p53 cysteine residues. Methyl seleninic acid (CH_3SeO_2H) and Na_2SeO_3 modulate p53 activity by phosphorylation	Smith et al. (2004)
	Selenodiglutathione also induces p53	Lanfear et al. (1994)
	Se compounds induced specific patterns of expression of gadd genes	Kaeck et al. (1997)
Inactivation of protein kinase C (PKC), a signalling receptor that plays a crucial role in tumour promotion by oxidants	Selective inactivation of PKC results from reaction of its catalytic domain with selenometabolites such CH_3SeO_2H (formed from membrane-bound CH_3SeH and fatty acid hydroperoxides), inhibiting tumour promotion and cell growth	Gopalakrishna & Gumimeda (2002)
Alteration in DNA methylation: abnormal methylation patterns are associated with neoplasia and inactivation of tumour-suppressor genes	Se affects the extent of DNA methylation and the activity of DNA methyl transferase	Davis et al. (2000), Davis & Uthus (2003), Fiala et al. (1998)
Blockage of the cell cycle: inhibits growth and may allow DNA repair to take place	CH_3SeH precursors can induce cell cycle arrest without single-strand breaks and with or without caspase induction and p53 regulation	Davis & Finley (2003)
	By contrast, selenite induces DNA single-and double-strand breaks, cell-cycle arrest, reduction in DNA synthesis and cell death, predominantly by necrosis	Medina et al. (2001)
Induction of apoptosis of cancer cells: generally involves the sequential activation of the caspases, a family of proteases capable of degrading cellular components	CH_3SeH precursors induce DNA double-strand breaks and cell death by apoptosis involving the caspase cascade	Medina et al. 2001, Unni et al. (2001), Wang et al. (2002), Davis & Finley (2003)
Inhibition of angiogenesis: new blood vessels are required for the growth and metastasis of tumours	CH_3SeH reduces microvessel density in chemically-induced rat mammary carcinomas (but not in normal tissue), the expression of vascular endothelial growth factor and matrix metalloproteinases	Jiang et al. (1999)
	p38 MAPK may be a key upstream mediator for the CH_3SeH-specific induction of vascular endothelial caspase-dependent apoptosis	Jiang et al. (2004)

SNP, single-nucleotide polymorphisms; SeMet, selenomethionine; MAPK, mitogen-activated protein kinase.
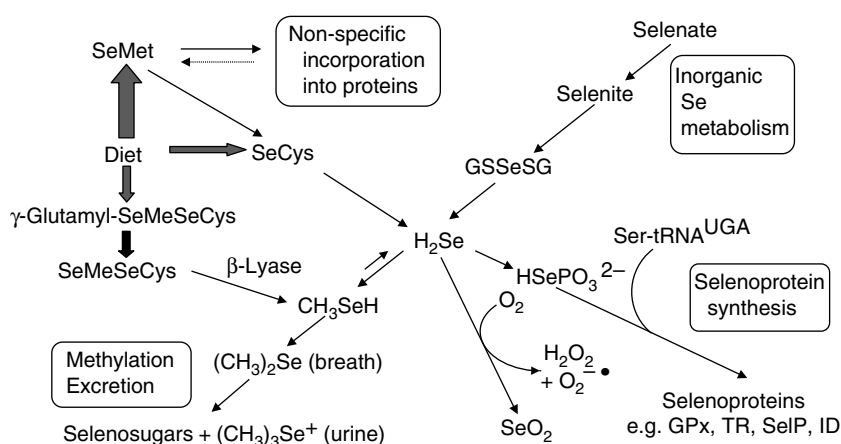
Fig. 2. The metabolism of dietary forms of selenium. SeMet, selenomethionine; SeCys, selenocysteine; SeMeSeCys, selenomethyl-SeCys; GSSeSG, selenodiglutathione; GPx, glutathione peroxidase; TR, thioredoxin reductases; SelP, selenoprotein P; ID, iodothyronine deiodinases (Adapted from Combs, 2001; Rayman, 2004.).

$0 \cdot 8$–$0 \cdot 9 \mu g/g$, as measured in dog toenails. Damage began to rise at $>1 \cdot 0 \mu g/g$ toenails, demonstrating the typical 'U'-shaped response to a nutrient that is toxic at high levels. Although the authors claim to have supplemented the dogs over the range of intake seen in US men, the baseline maintenance diet, at $0 \cdot 3 \mu g$ Se/g, gave an intake in the control group of $6 \mu g/kg$ body weight, already equivalent to a high human intake, i.e. $450 \mu g/d$ for a 75 kg man. The highest supplement level was an additional $6 \mu g$ Se/kg body weight, equivalent to a total daily intake of $900 \mu g/d$ for a 75 kg man. It is not surprising, therefore, that the upward arm of the 'U'-shaped response was breached.

In a New Zealand study of men aged 50–75 years at risk of prostate cancer (prostate-specific antigen >4 ng/ml), the comet assay was reported to show a significant ($P = 0 \cdot 02$) inverse relationship with overall accumulated DNA damage in blood leucocytes from subjects with serum Se levels below the mean (Karunasinghe et al. 2004). As the mean serum Se was measured as 98 (SD 17) $\mu g/l$, this finding suggests that serum levels $>98 \mu g/l$ are required for the prevention of DNA damage in New Zealand men.

Women born with a BRCA1 mutation carry a lifetime risk of breast cancer of 80% and a lifetime risk of ovarian cancer of 40% (Kowalska et al. 2005). The BRCA1 gene product is involved in maintaining the integrity of the human genome and helps repair double-strand breaks. When blood lymphocytes from BRCA1 carriers are exposed to bleomycin, a known mutagen that induces double-strand breaks, an increased frequency of chromosome breaks per cell occurs, i.e. $0 \cdot 58$ in BRCA1 carriers v. $0 \cdot 39$ in non-carriers (Kowalska et al. 2005). In thirty-two female BRCA1 carriers supplemented with Se ($276 \mu g$ as Na$_2$SeO$_3$/d) for 1–3 months, the frequency of chromosome breaks was found to be reduced from $0 \cdot 63$ per cell before supplementation with Se to $0 \cdot 40$ per cell after supplementation, bringing it to the level in non-carrier controls. Thus, Se may have the potential to reduce breast cancer risk in these women.

Reduction of oxidative stress

The modification of the anti-cancer effects of Se by other antioxidant nutrients suggests that the ability of Se in selenoproteins to reduce oxidative stress is relevant to its anti-cancer effects. Thus, Se intake or status becomes more important when the concentration of other antioxidants or the activity of other antioxidant enzymes is low. The strongest effect of Se on cancer risk has been shown among those subjects with the lowest levels of dietary antioxidant vitamins and carotenoids (Willett et al. 1983; Salonen et al. 1985; Kok et al. 1987; Knekt et al. 1990; van den Brandt et al. 1993, 2003; Yu et al. 1999), and particularly at low α-tocopherol concentrations (Combs & Gray, 1998). In the study of Yoshizawa et al. (1998), summarised in Table 2, the inverse association between Se status and advanced prostate cancer was found to be slightly stronger after excluding men with an intake of vitamin E >30 mg/d, mostly from supplementary sources (OR $0 \cdot 29$ v. $0 \cdot 35$). Data, as yet unpublished, from the NPC trial (M Reid, personal communication) show that the effect of Se supplementation on prostate cancer risk only reaches significance in subjects in the bottom half of α-tocopherol status, i.e. plasma concentrations $<21 \cdot 66 \mu M$ ($P = 0 \cdot 03$ v. $P = 0 \cdot 31$ in the top half of α-tocopherol status).

A further indication of a link between the antioxidant capacity of Se and cancer risk is seen in the modification of the Se-dependent risk by a polymorphism in Mn superoxide dismutase (MnSOD), the primary antioxidant enzyme in mitochondria. MnSOD has an Ala/Val polymorphism at codon 16 in the mitochondrial targetting sequence that affects the structure of the protein. The relationship between prostate cancer, the MnSOD polymorphism and baseline plasma Se concentration has been investigated in 567 cases and 764 controls nested within the prospective Physicians' Health Study (Li et al. 2005). Although little overall association was found between MnSOD polymorphism and prostate cancer risk, in men with the Ala/Ala genotype high Se status (4th quartile v.

Table 6. Association between glutathione peroxidase Pro198Leu allele and cancer risk (odds ratio; OR) and modification of risk by manganese superoxide dismutase (MnSOD) genotype

Cancer	Tissue sampled	SNP genotype	OR*	95% CI	Reference
Lung	Blood	Pro/Leu	1·8	1·2,2·8	Ratnasinghe et al. (2000)
		Leu/Leu	2·3	1·3,3·8	
Bladder	Blood	Pro/Leu	2·6	1·5,4·8	Ichimura et al. (2004)
		+MnSOD Val/Ala+ Ala/Ala	6·3	1·3,31·2	
Breast	Blood	Pro/Leu	0·9	0·7,1·2	Knight et al. (2004)
		Leu/Leu	0·8	0·5,1·3	

SNP, single-nucleotide polymorphism.
*Compared with Pro/Pro genotype.

1st quartile) was shown to be associated with a significantly lower risk (RR 0·3 (95% CI 0·2, 0·7); $P = 0.002$ for trend). For clinically-aggressive prostate cancer the RR was shown to be even more reduced (0·2 (95% CI 0·1, 0·5), $P < 0.001$ for trend). In contrast, in men with one or two Val alleles the RR in the 4th quartile compared with the 1st quartile was shown to be less affected by Se status (0·6 (95% CI 0·4, 1·0) and 0·7 (95% CI 0·4, 1·2) for total and clinically-aggressive prostate cancer respectively; Li et al. 2005). The interdependence of MnSOD, Se status and prostate cancer risk implies a role for the antioxidant selenoenzymes.

Evidence from selenoprotein genotype data for a role of selenoproteins in cancer prevention

It had been thought that selenoenzymes were not involved in anti-cancer mechanisms because the level of Se supplementation that reduced cancer risk (200 μg/d) was greater than the amount then believed to be needed to optimise selenoenzyme activity (Combs & Gray, 1998). However, it has recently become clear that optimal expression of some selenoproteins, notably selenoprotein P, requires a higher amount, as yet undetermined, of dietary Se (Xia et al. 2005) and, furthermore, that a substantial number of individuals may have a higher requirement for Se for efficient synthesis of selenoproteins, as will be explained later (p. 537).

Individuals differ substantially in their ability to increase selenoprotein activity in response to additional dietary Se (Brown et al. 2000). This inter-individual variation in selenoprotein expression levels may be accounted for by SNPs in selenoprotein genes that determine the efficiency with which individuals can incorporate Se into selenoproteins (Kumaraswamy et al. 2000; Ratnasinghe et al. 2000; Hu et al. 2001; Hu & Diamond, 2003). Thus, requirements for dietary Se for optimal protection against cancer may be much higher in individuals carrying particular functional selenoprotein SNPs such as those that will be described.

Cytosolic glutathione peroxidase. Recent studies have reported a link between cancer risk and polymorphisms in the cytosolic GPx selenoprotein (*GPx1*) gene at Pro198-Leu. Possession of the Leu198 allele has been found to be associated with an increased risk of lung cancer in Caucasians but not among ethnic Chinese, who do not appear to show this polymorphism (Ratnasinghe et al. 2000). Possession of the Leu198 allele also confers an increased risk of bladder cancer (see Table 6) and that risk is further raised in men who have one or two Ala alleles at codon 9 (apparently identical to codon 16, as described earlier) in exon 2 of MnSOD (Ichimura et al. 2004). In the 213 patients with bladder cancer, when compared with the Pro/Pro genotype, the Pro/Leu genotype was found to be significantly associated with advanced tumour stage (OR 2·58 (95% CI 1·07, 6·18); $P = 0.034$ for tumour stage T2–4 v. tumour stage Ta+1; Ichimura et al. 2004). By contrast, in a case–control study of 399 cases of incident invasive breast cancer and 372 controls, no association between breast cancer and GPx1 Pro198Leu was found (Knight et al. 2004). However, the allele of *GPx1* containing four GCG repeats was found to be significantly associated with breast cancer risk in premenopausal women (OR 1·55 (95% CI 1·04, 2·30) for carriers v. non-carriers). Importantly, *GPx1* with the Leu allele has been shown to be less responsive to stimulation of its enzyme activity by Se supplementation than *GPx1* with the Pro allele (Hu & Diamond, 2003).

Studies showing selective loss of the Pro198 allele of the *GPx1* gene during tumour development, as detected by loss of heterozygosity at this locus, implicate GPx1 in the risk and development of tumours. The Leu/Leu genotype has been found to be almost twice as common in DNA from breast cancer tissue as it is in DNA from cancer-free individuals, while the Pro/Leu genotype was found to be underrepresented, indicating loss of heterozygosity at this locus in breast tumour development (Hu & Diamond, 2003). Similarly, DNA samples from head and neck tumours exhibit fewer heterozygotes and an increased frequency of the Leu/Leu genotype compared with DNA from the cancer-free population (Hu et al. 2004).

15 kDa selenoprotein. Sep15 is expressed at high levels in normal liver and prostate but at reduced levels in the corresponding malignant organs (Behne et al. 1997). It is located in the endoplasmic reticulum, tightly complexed to UDP-glucose:glycoprotein glucosyltransferase, an enzyme involved in the quality control of protein folding (Korotkov et al. 2001). (The location of Sep15 may be of interest as some forms of Se appear to activate endoplasmic reticulum stress-induced apoptosis, as mentioned earlier.) The *Sep15* gene lies on chromosome 1p22.3 at a locus commonly deleted or mutated in human cancers (Kumaraswamy et al. 2000; Kryukov et al. 2003). Two SNPs at positions 811 (C/T) and 1125 (G/A) that are in strong allelic association have been studied in the 3′-UTR of the *Sep15* gene; G1125A lies within a functional SECIS element (Kumaraswamy et al. 2000). The T811/A1125 variant has been shown to be more effective in supporting UGA read-through than the C811/G1125 variant, but less responsive to the addition of Se to the culture medium (Hu et al. 2001; Kumaraswamy et al. 2000). Thus, the identity of the nucleotides at 811 and 1125 influences the function of the Sep15 SECIS element in a Se-dependent

manner (Kumaraswamy *et al.* 2000). Individuals possessing one or other of these haplotypes may therefore differ in the efficiency with which they can make Sep15 and in how well they can use dietary Se.

The frequency of the T811/A1125 haplotype is 0·25 in Caucasians and 0·57 in African Americans, who have a higher incidence of prostate cancer (Hu *et al.* 2001). If lower levels of the *Sep15* gene product predispose cells to malignant transformation in the human population, then those individuals carrying a particular *Sep15* gene polymorphism may be at a greater risk of cancer and might require a higher Se intake for protection. Furthermore, a difference was found among African Americans (but not Caucasians) in allele frequencies in DNA from breast or head and neck tumours compared with DNA from cancer-free controls. The authors (Hu *et al.* 2001; Diwadkar-Navsariwala & Diamond, 2004) suggest that this difference is likely to be largely related to loss of heterozygosity at the *Sep15* locus.

Additional evidence for an effect of this polymorphism on cancer risk comes from a study of Apostolou *et al.* (2004), which has shown that the A1125 variant of *Sep15* is less responsive to the apoptotic and growth-inhibitory effects of Se than the G1125 variant. The *Sep15* gene was shown to be down-regulated in 60% of malignant-mesothelioma cell lines and tumour specimens in this study.

Phospholipid glutathione peroxidase. Phospholipid GPx (Gpx4) decreases lipid hydroperoxide levels, and thus inhibits the lipoxygenases that metabolise arachidonic acid to generate intermediates that mediate signals for increasing cell proliferation and inhibiting apoptosis (Kim & Milner, 2001). In particular, it inhibits 5-lipoxygenase and reduces the production of 5-hydroxyeicosatetraenoic acid, which is known to stimulate the proliferation of prostate cancer cells (Ghosh & Myers, 1998). Inhibition of 5-lipoxygenase has been shown to trigger massive apoptosis in human prostate cancer cells (Ghosh & Myers, 1998). The C718 allele of the *GPx4* T718C SNP, which is close to the SECIS element in the $3'$-UTR, has a frequency of 0·45 in Caucasians and is associated with increased levels of lymphocyte 5-lipoxygenase total products (Villette *et al.* 2002). Thus, this polymorphism has functional consequences and may influence the production of 5-hydroxyeicosatetraenoic acid and consequently the proliferation or apoptosis of prostate cancer cells (Villette *et al.* 2002). Two genetic studies (Hsieh *et al.* 2001; Wiklund *et al.* 2003) have shown linkage of the chromosome 19p13·3 region that contains the *GPx4* gene to prostate cancer.

Selenoprotein P. SNPs have also been identified in selenoprotein P, a selenoprotein believed to be involved both in protection from reactive oxygen and nitrogen species and in the transport of Se to tissues. Normally, the selenoprotein P gene is highly expressed in prostatic epithelium but it is down regulated in a subset of human prostate tumours, mouse tumours and prostate carcinoma cell lines (Calvo *et al.* 2002). Calvo *et al.* (2002) have suggested that reduced selenoprotein P synthesis occurs in a subset of patients resulting in loss of protection from oxidative stress.

Likelihood of simultaneous and consecutive effects at different cancer stages

Given the breadth of evidence for the involvement of forms of Se in various anti-cancer processes, it is likely that Se acts at a number of stages in cancer development and by a number of different mechanisms that may operate simultaneously, or consecutively, involving both small-molecular-weight Se metabolites and selenoproteins. Diwadkar-Navsariwala & Diamond (2004) have proposed a model in which the likelihood of cancer development is linked to reduced levels of one or more protective selenoproteins resulting from (1) inadequate dietary Se intake and/or (2) genetic polymorphisms that result in an increased Se requirement for selenoprotein synthesis and/or (3) allelic loss of one or two gene copies during tumour development. It may even be that exposure to some forms of Se provokes cellular stress, up-regulating protective response systems (such as glutathione-S-transferase) that reduce cancer risk (V Gladyshev, personal communication). Clearly, this very complex area is far from being fully understood.

Effect of selenium on progression and metastasis

There are a few indications that Se can have an effect on cancer progression or metastasis. Three examples are: (1) the effect of Se status on prostate cancer is greater for advanced disease (disease that has spread beyond the prostate) than for primary disease (Nomura *et al.* 2000; van den Brandt *et al.* 2003; Li *et al.* 2004), suggesting an inhibitory effect on tumour spread; (2) angiogenesis is required for progression and metastasis. It requires growth factors such as vascular endothelial growth factor and proteolytic degradation of the extracellular matrix by the family of matrix metalloproteinases. Vascular endothelial growth factor expression and protein levels are significantly lowered, as is the activity of matrix metalloproteinases by CH_3SeH precursors (Jiang *et al.* 1999, 2000, 2004), while selenite inhibits invasion of human fibrosarcoma cells by reducing the expression of matrix metalloproteinase-2 and -9 (Yoon *et al.* 2001); (3) the tumour stage of bladder cancer is affected by GPx1 genotype, giving indirect evidence that GPx1 is relevant to bladder cancer progression (Ichimura *et al.* 2004).

Current and future selenium-cancer projects

The Selenium and Vitamin E Cancer Prevention Trial (SELECT), sponsored by the National Cancer Institute at a cost of US$180 × million, is a phase III randomised double-blind placebo-controlled trial designed to test the efficacy of Se (200 µg L-selenomethionine) and vitamin E (400 mg DL-α-tocopherol), both alone and in combination, in the prevention of prostate cancer (Klein, 2004). The target accrual of 32 400 male volunteers has been achieved and final results are expected in 2013.

The possibility of raising even one-tenth of the sum made available in the USA for the Selenium and Vitamin E

Cancer Prevention Trial for a similar-scale trial in Europe is remote. However, European investigators are still hopeful that a sufficient sum can be raised to carry out a less-expensive web-based trial (Prevention of Cancer by Intervention with Selenium) with Se-enriched yeast in Europe where Se intakes and status are so much lower. As the strongest treatment effect in the NPC Trial has been observed in subjects in the lowest tertile of plasma Se at baseline (Duffield-Lillico *et al.* 2002), Se intervention in European subjects would greatly increase the chance of seeing an effect. Equally importantly, it would eliminate the possibility of adverse effects in individuals of already-adequate Se status ($\geq 120 \mu g/l$) such as were seen in the top tertile in the NPC Trial (Duffield-Lillico *et al.* 2002). Furthermore, women as well as men would be included in the European trial.

Se-enriched yeast is currently being used in further prostate cancer studies at the Arizona Cancer Center at doses of 200–800 µg/d, i.e. the Negative Biopsy Trial (Stratton *et al.* 2003*a*), the Preprostatectomy Trial (Marshall, 2001) and the Watchful Waiting Trial (Stratton *et al.* 2003*b*).

There has not yet been a human trial with SeMe-SeCys, although preparation for such a study in human subjects by Ip and colleagues is apparently underway (M Reid, personal communication). As SeMe-SeCys is not a very good precursor for selenoproteins, the results of such a study would be very informative.

The present author and colleagues are investigating the effect of functional selenoprotein SNPs on prostate cancer risk using DNA samples from 1400 prostate cancer cases and 800 age- and location-matched controls from the Swedish prostate cancer study (Wiklund *et al.* 2003). Careful speciation work (Goenaga-Infante *et al.* 2004, 2005) is also being extended to identify low-molecular-weight Se species in body tissues and fluids and in Se-enriched yeast and plants.

Will industry allow us to find the definitive answer?

Much time has elapsed during which scientists have spent increasing amounts of time and effort in fund-raising for demanding and meticulous studies to clarify whether Se truly has an effect in reducing cancer risk. Industry has already made up its mind and is not prepared to wait. Apart from Se supplements that have been available for many years, there is now a greater push towards Se-containing functional foods and fertilisers and the selection or breeding of high-Se crop varieties (Broadley *et al.* 2006). The worry is that population-based studies will become increasingly difficult to carry out under these circumstances, so that the answer on Se and cancer in populations may never be definitive unless a European-based trial can be prioritised.

Acknowledgements

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