We appreciate the opportunity to address the comments of Krawczak and Cooper published in the 'Discussion forum' in Mutagenesis, 13, 319-320 (1998).

Krawczak and Cooper take issue with the validity of the conclusions drawn from our study on the relationship between the distribution of adducts of the polycyclic aromatic hydrocarbon (PAH) benzo[a]pyrene (B[a]P) and lung cancer mutations in the p53 gene (Denissenko et al., 1996). We address their points as follows. They conclude that the p53 mutational spectrum in lung cancer is not much different from other cancers. This statement, on which most of their argument is based, is erroneous and misleading. There is no doubt that the p53 mutational spectrum (i.e. the distribution of mutations along the gene) and the mutational signature (i.e. the characteristic ratio of transitions, transversions, deletions, etc.) is different between lung cancer and other cancer types (Hernandez-Boussard and Hainaut, 1998). In lung cancer, ~40% of the mutations are G→T transversions, >90% of them biased to a guanine on the non-transcribed DNA strand (Greenblatt et al., 1994). This type of mutation is rare in most other cancers. In addition, there is a striking scarcity of transition mutations at CpG sequences in the p53 gene of lung cancer (9%), despite methylation of all CpG sites in lung cells (Tornaletti and Pfeifer, 1995). Transition mutations at CpG are much more frequent in almost all other cancers or in the germline (up to 50%) and have been linked to deamination of endogenous 5-methylcytosine bases (Gonzalgo and Jones, 1997). Thus, as is widely recognized (Greenblatt et al., 1994; Hernandez-Boussard and Hainaut, 1998) but simply denied by Cooper and Krawczak, the mutational signature in lung cancer clearly suggests the involvement of exogenous carcinogens. The cigarette smoke component B[a]P or similar PAHs are strong candidates for being involved in p53 mutagenesis, since these highly carcinogenic compounds cause predominantly G→T transversions with a strand bias in selectable genes of cultured human cells or in animals (Greenblatt et al., 1994). From the several mutational hotspots that are selectively damaged by activated B[a]P (Denissenko et al., 1996), codon 157 is a hotspot unique to lung cancer. Mutations occur frequently at codons 248 and 273 also in other cancers and are usually recovered there as transition mutations at the corresponding CpG sequences (Hainaut et al., 1997). In lung cancers, however, G→T transversions predominate at these mutational hotspots. Although selection certainly plays a role in shaping the p53 mutational spectra in all cancers, there are many codons (~150 in lung cancer and ~220 in all cancers combined) that can be targets of different missense mutations (Hainaut et al., 1997). The striking coincidence of the lung cancer mutational spectrum in smokers and the B[a]P adduct spectrum (Denissenko et al., 1996, 1998), together with the dominance of G→T transversion mutations in these cancers, suggests that a large proportion of p53 mutations in lung cancer are not caused by endogenous processes, as implied by Krawczak and Cooper (1998), but are caused by carcinogens of the PAH class found in cigarette smoke.

Cooper and Krawczak criticize us for not including data on mutations in non-smokers. About 90% of human lung cancers are associated with smoking. p53 lung cancer mutations are generally more common in smokers than in non-smokers and the frequency of G→T transversions on the non-transcribed
strand is positively correlated with lifetime cigarette consumption (Suzuki et al., 1992). As recognized previously (Greenblatt et al., 1994), the mutational signature is very different between smokers and non-smokers. Only very few G→T mutations have been found in lung cancers from non-smokers (Takeshima et al., 1993; Takagi et al., 1995, 1998; Hernandez-Boussard and Hainaut, 1998). Despite the limited number of p53 mutations in lung cancers of non-smokers, the distribution of mutations along the gene is clearly different from that in smokers and also is different from that in lung cancers associated with radon exposure (Figure 1). The origin of the mutational hotspot at codon 249 in radon-associated lung cancers is controversial (Taylor et al., 1994; Venitt and Biggs, 1994; Hei et al., 1994; Bartsch et al., 1995; Lo et al., 1995).

Ninety five percent of G→T transversions in lung cancer can be ascribed to guanines on the non-transcribed DNA strand (Greenblatt et al., 1994). Slow repair of PAH–guanine adducts on the non-transcribed strand (including the mutational hotspots) is additional strong evidence that these mutations are caused by polycyclic aromatic compounds such as B[a]P found in cigarette smoke (Denissenko et al., 1998). Smokers are chronically exposed. It is expected that continuous exposure, together with slow absorption of PAHs into the tracheal epithelium and extensive metabolism (Gerde et al., 1997), provides sufficiently high DNA adduct levels. Sequence-selective adduct formation and poor repair of the non-transcribed strand contribute strongly to shape the mutational profile of the p53 gene in lung cancer.

In conclusion, none of the objections raised by Krawczak and Cooper can be substantiated and our conclusions remain as initially reported (Denissenko et al., 1996).

References


Gerd P.Pfeifer1, Mikhail F.Denissenko1 and Moon-shong Tang2

1Beckman Research Institute of the City of Hope, Department of Biology, Duarte, CA 91010 and 2University of Texas M.D. Anderson Cancer Center, Science Park, Smithville, TX 78957, USA.