Genes and Environment, Vol. 28, No. 1 pp. 38–39 (2006)

Letter to editor

Understanding of Genotoxic Mechanism of Action for Carcinogen Risk Assessment to Humans; a Commentary to the Discussion at the 4th International Workshop on Genotoxicity Testing (IWGT)

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(Received December 9, 2005; Accepted January 6, 2006)

At the 4th International Workshop on Genotoxicity Testing (IWGT) held on September 9–10, 2005 at San Francisco, USA, about 180 participants from government, industry and academia mainly in the USA, EU, Canada and Japan discussed some issues concerning two testing methods, i.e. *in vivo* Comet test and *in vivo* micronucleus test, and four strategies, i.e. follow-up on *in vitro* positives, metabolic considerations, genotoxic or non-genotoxic tumor mechanisms, and unique or non-relevant *in vivo* positives. In the method sub-working groups, *in vivo* Comet test and *in vivo* micronucleus test (non-erythrocytes) were focused, because of importance of establishment of the methods to detect *in vivo* genotoxicity in organs other than bone marrow. I joined each sub-working group of testing methods and strategies, i.e. Comet test and genotoxic or non-genotoxic tumor mechanisms, with Japanese colleagues. In the genotoxic or non-genotoxic tumor mechanism sub-working group in the strategy topics, three groups including us reported some carcinogen case studies. We also proposed a decision tree to estimate the mode of action of carcinogenesis. Although the discussion details will be published soon as consensus reports, here I would like to briefly introduce my concerns through discussion in the strategy sub-working group.

Carcinogen risk is recently assessed with a weight of evidence approach based on the mechanism of action in carcinogenesis basically in accordance with EPA guidelines for carcinogen risk assessment (1). Members of the strategy sub-working group discussed an issue entitled ''testing required to exclude a genotoxic mechanism of action when carcinogenesis studies yield significant tumor findings''. It is believed that the carcinogenic mechanism of a carcinogen would be unrelated to its genotoxicity including mutagenicity when the compound clearly shows negative results in a standard battery of genotoxicity testing, i.e. bacterial reverse mutation test, mammalian cell cytogenetic test and *in vivo* rodent micronucleus test. However, some compounds increase the significant number of tumors in rodent bioassays with no clear evidence of non-genotoxic mechanisms of action such as cell proliferation and inhibition of apoptosis, and, in such cases, some additional genotoxicity testing may offer helpful information to understand the mechanisms of action of their carcinogenicity (2). The sub-working group members reached a consensus that *in vivo* target-organ studies would contribute to comprehend the mechanisms of action, and the following tests are primarily recommended: Comet or alkalin-elution test, DNA adduct test, liver UDS test, transgenic rodent gene mutation test and micronucleus test with non-erythrocytes. The group members also indicated that a structure-activity relationship of a compound compared to other carcinogens should be considered to understand the mechanisms of action, and analysis of the structure-activity relationship should also include its (organ-specific) metabolites. Finally, based on the information from these additional studies, a weight of evidence approach will be applied to determine whether tumors at each target site and each tumor characteristic may be related to genotoxic mechanisms of action or non-genotoxic mechanisms of action, e.g. if the standard battery and additional target-organ genotoxicity studies show clearly negative results with a carcinogen and there is no doubtful structure-activity relationship of the carcinogen and its all metabolites, we consider that the tumor may be induced by an unknown non-genotoxic mechanism of action.

This decision seems quite sufficient from the regulatory science aspect but questions have arisen from the

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basic science aspect, i.e. ''Can we estimate the genotoxic mechanisms of action of chemical carcinogenesis with the above recommended tests?'' and more essentially ''Can we understand the true relationship between genotoxic mechanisms of action and carcinogenic mechanisms of action with genotoxicity testing results?'' Carcinogenesis is generally considered a result of sequential changes of some critical proto-oncogenes and/or tumor suppressor genes. Genotoxicity tests can detect DNA damage, gene mutation and/or chromosome mutation induced by compounds, and thus it is practically recognized that a carcinogenic compound with a significant positive finding indicates a genotoxic mechanism of action in its carcinogenicity. However, there are some difficulties and limitations to extrapolate the positive results of genotoxicity tests shown in a carcinogen to its genotoxic roles in significant tumor production, because 1) experimental designs and conditions of genotoxicity tests are usually specialized to increase sensitivity, especially *in vitro* tests, 2) alterative target genes/chromosomes are examined in genotoxicity tests, which are different from true target genes/chromosomes in carcinogenesis, 3) DNA damage may be repaired and cells suffering DNA damage may be excluded by necrosis and/or apoptosis. Originally, genotoxicity tests were developed and refined to detect genotoxins sensitively, and thus the objectives of these tests would be primarily limited to hazard identification and screening of genotoxins; however this limitation is sometimes forgotten and we often simply decide that the carcinogenicity of a compound is related to its genotoxic mechanisms of action when positive results are obtained in genotoxicity tests. We need to realize that significant differences may exist between positive findings in genotoxicity tests and genotoxic mechanisms of action in carcinogenesis (3).

How can we understand the roles of genotoxicity in carcinogenesis? One approach would be the precise analysis of mechanisms of action of genotoxicity. When a carcinogenic compound shows genotoxicity in an *in vitro* test such as a bacterial reverse mutation test, in some cases, it is concluded that the compound is a genotoxic carcinogen, or in some cases, further studies are performed to clarify whether the *in vitro* positive result is relevant to *in vivo* genotoxicity and carcinogenicity. Usually, however, we do not analyze the mechanism of why the compound showed a positive result in the *in vitro* test and do not assess the carcinogen risk based on the mechanism. Even when the outputs as positive responses are quite similar, mechanisms of action may be quite different, e.g. 1) direct DNA reactive effects of compounds, 2) non-DNA reactive effects of compounds such as topoisomerase inhibitors, 3) increase in intracellular active oxygen species by compound effects, and we notice that carcinogen risk may be different among these three examples. Even if the mechanism of action is clarified as a direct DNA reactive effect of compounds, we should decide whether the effects are actually relevant to human carcinogenesis. Such precise analysis of mechanisms of action would offer helpful information to assess human risk correctly. One of our JEMS objectives should be not only to detect potent human genotoxins and carcinogens but also to estimate their human risk based on the mechanisms of action of positive findings (4,5).

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