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Key Characteristics Approach to Carcinogenic Hazard Identification

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ABSTRACT: Evaluating carcinogenic mechanisms is a challenging part of hazard identification, as mechanistic data are both voluminous and diverse. An evaluation approach based on 10 key characteristics of human carcinogens provides a holistic and unbiased way to tackle this challenge.

Mechanistic and other relevant data may provide evidence of carcinogenicity and help assess the relevance and importance of findings of cancer in animals and in humans.[1] A review of Group 1 human carcinogens classified in *IARC Monographs* Volumes 1−99 revealed several challenges for conducting a comprehensive evaluation of mechanistic data to support identification of carcinogens.[2] First, human carcinogens share one or more characteristics that are related to the mechanisms by which agents cause cancer. Second, each different human carcinogen may exhibit a different spectrum of these key characteristics (KCs) of carcinogens and may operate through distinct mechanisms. Third, for many carcinogens evaluated prior to Volume 100, few data were available on some mechanisms of recognized importance in carcinogenesis, such as epigenetic alterations. Fourth, there was no widely accepted method to search systematically for relevant mechanistic evidence, resulting in some variation in the mechanistic topics addressed across IARC evaluations.

To address these challenges, a set of KCs of human carcinogens was recently introduced to form a common basis for assembling and evaluating mechanistic evidence to support cancer hazard identification.[2,3] The KCs comprise the chemical and biological properties of known human carcinogens (see Figure 1) and are therefore distinct from the hallmarks of cancer that relate to the properties of tumors and cancer cells.[3] One intriguing aspect of using the KCs to assemble data relevant to carcinogenic mechanisms is that an *a priori* hypothesis about the mechanism of action is not required. Instead, the KCs are based on empirical observations of characteristics associated with known carcinogens and thus provide an unbiased survey of the mechanistic literature. As such, the approach based on the KCs, outlined below, "avoids a narrow focus on specific pathways and hypotheses and provides for a broad, holistic consideration of the mechanistic evidence".[4]

The KCs have been applied in mechanistic data evaluations for more than 30 mechanistically diverse chemicals and complex exposures, classified into Groups 1, 2A, 2B, and 3 by *IARC Monographs* expert Working Groups since 2015.[5] This experience demonstrated several strengths, such as the ability to identify gaps in evidence across the agents evaluated. Indeed, the approach revealed a broad literature encompassing multiple KCs for Groups 1 and 2A carcinogens identified, in contrast to many fewer studies available for Groups 2B and 3 agents. Mechanistic data were used as part of the overall evaluation to classify two substances (tetrabromobisphenol A and tetrachloroazobenzene) in Group 2A, both of which modulate receptor-mediated effects in combination with other KCs. Other authoritative bodies are increasingly using the KCs, such as was done in recent evaluations of antimony trioxide and six haloacetic acids by the National Toxicology Program's Report on Carcinogens.

As the KC approach has been applied, several opportunities for refinement have been identified and are the subject of ongoing efforts. Differences in the relative importance among KCs have become apparent, leading to challenges in interpreting evidence on individual KCs. Indeed, some human carcinogens exhibit a single or primary KC that is influential in their cancer hazard classifications. For instance, ethylene oxide is genotoxic, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin modulates receptor-mediated effects, and etoposide alters DNA repair. Similarly, oncogenic viruses cause immortalization, and certain drugs are, by design, immunosuppressive. Because of their greater specificity of association with carcinogens, emphasis is given to these KCs in Figure 1. At the other extreme, non-carcinogens can also induce oxidative stress, and so this type of KC should be interpreted with caution unless found in combination with other KCs.[5] In such cases, evidence on a group of KCs, in concert, has strengthened mechanistic conclusions.

Efforts are also ongoing to refine the literature search filters and inclusion criteria for the KCs to more clearly highlight studies with the greatest importance to carcinogenicity. For instance, regarding "induces oxidative stress", evidence of oxidative damage to DNA was accorded greater weight than other types of oxidative damage. Moreover, conclusions were strengthened by experiments showing attenuation by anti-
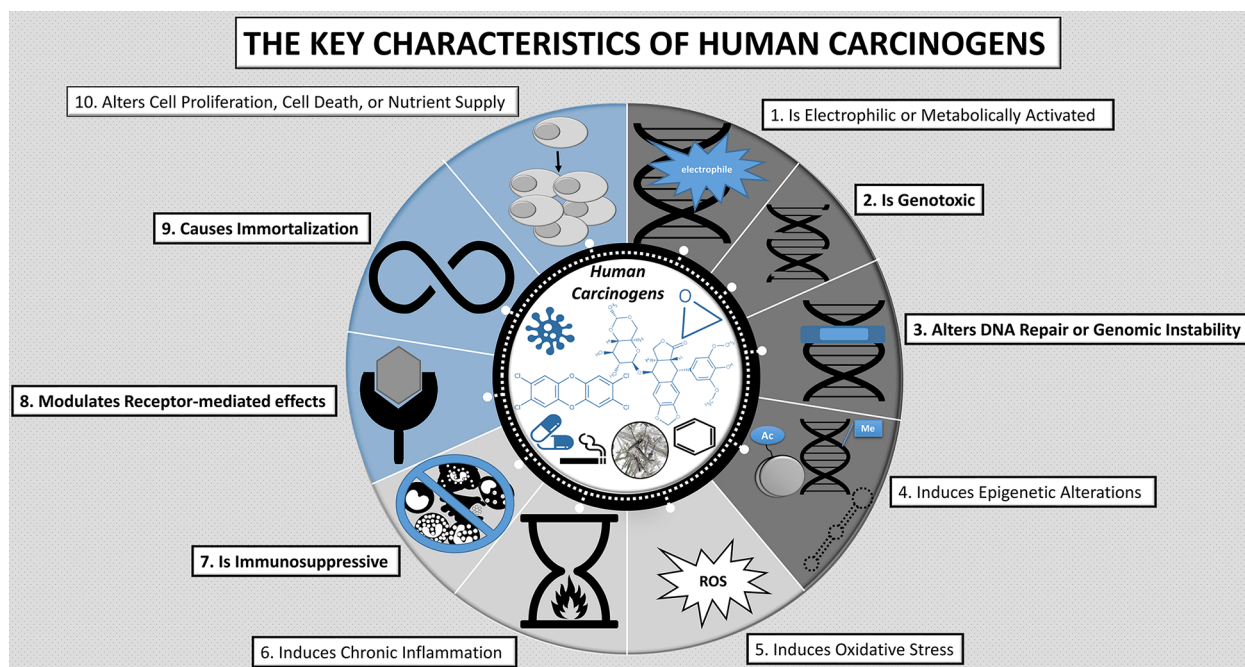
Figure 1. Key characteristics (KCs) of human carcinogens. Some human carcinogens such as ethylene oxide, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and etoposide (shown in blue in the center of the figure) exhibit a single or primary KC that has alone been influential in cancer hazard classifications. Other human carcinogens such as benzene, cigarette smoke, and asbestos (shown in black in the center of the figure) are recognized as exhibiting several KCs.

oxidants of effects relevant to the KCs or enhancement of carcinogenicity in relevant knockout animals (e.g., $Nrf2^{-/-}$ mice).[5] Regarding other KCs, studies showing chronic inflammation *in vivo* (for KC#6), functional immune suppression and decreased NK cell activity (for KC#7), and inhibition of apoptosis, sustained proliferation, and hyperplasia (for KC#10) have also been given greater prominence. This experience will aid future expert evaluations of both data-rich and data-poor agents as the approach is taken forward.

Further work is focused on applying knowledge about the KCs together with gene-association data and biological pathway information from publicly available and open-source resources (e.g., the Comparative Toxicogenomics Database and WikiPathways) with the purpose of improving carcinogenicity predictions and allowing for priority-setting of carcinogen evaluations. The linking and tagging of KCs with the biological pathways on the WikiPathways Web site and a gene set enrichment analysis tool will facilitate open-source screening of future "omics" data to reveal when compounds of unknown carcinogenic potential have strong association with carcinogens.

Finally, although the KCs relate to carcinogens and are distinct from the hallmarks of cancer that relate to cancer cells, collaborative work is pursuing opportunities to integrate the KCs of carcinogens and the hallmarks of cancer. This is expected to help identify and develop *in vitro* assays and *in vivo* biomarkers for all the KCs and their associated hallmarks (a CarciCAST, enhancement of ToxCAST) and to anchor the KCs in a biological outcome.

In summary, application of the KCs to hazard identification is a robust approach that complements other efforts to advance identification of the causes of human cancer, the first step in cancer prevention. The *IARC Monographs* have successfully used this approach since 2015, and other agencies worldwide are beginning to apply it. Experience to date has revealed substantial variability in the extent of mechanistic information available across agents and across KCs, which can help inform research priorities. Potential refinements are being explored to further improve the evaluation of mechanistic data to support identification of human carcinogenic agents. In parallel, progress is advancing on development of KCs for other toxicological hazards, including endocrine disruption and reproductive toxicity.[4]

■ **AUTHOR INFORMATION**

Notes

Views expressed in this publication are those of the authors and not necessarily the views of the ACS nor the European Commission.

The authors declare the following competing financial interest(s): M.T.S. has served as a consultant and expert witness in U.S. litigation involving chemical and pharmaceutical exposures and various disease outcomes, including neuropathies and cancer.

■ **ACKNOWLEDGMENTS**

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