This is a background paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced

CC/2020/05

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC).

Position paper – The Tumour Microenvironment; draft v 0.a

1. A scoping paper (CC/2020/01) was presented at the March 2020 COC meeting outlining aspects of the tumour microenvironment and the possible interaction with environmental chemicals. It was decided that the Committee should have a position paper on the topic and a draft is attached here for consideration.

Questions for the Committee

2. Members are asked to consider this draft position paper and in particular:

   i. Are there any aspects Members are aware of that are not covered by the paper?

   ii. Is the Committee content to publish this position paper?

NCET at WRc/IEH-C under contract supporting the PHE COC Secretariat
July 2020

This is a draft position paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced.

Committee on CARCINOGENICITY

Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC)

COC Position Paper

The Tumour Microenvironment

https://www.gov.uk/government/groups/committee-on-carcinogenicity-of-chemicals-in-food-consumer-products-and-the-environment-coc

COC Secretariat
c/o Public Health England
Centre for Radiation, Chemical and Environmental Hazards
Chilton, Didcot, Oxfordshire OX11 0RQ
© Crown copyright 2020

You may re-use this information (excluding logos) free of charge in any format or medium, under the terms of the Open Government Licence v3.0. To view this licence, visit OGL or email psi@nationalarchives.gsi.gov.uk. Where we have identified any third party copyright information you will need to obtain permission from the copyright holders concerned. Any enquiries regarding this publication should be sent to COC@phe.gov.uk.

This is a draft position paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced.

Committee on CARCINOGENICITY

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COC)

Position Paper - The Tumour Microenvironment – draft v0.a

Introduction

1. The history of cancer research has shown that cancer development is driven by both genetic and epigenetic events. Recent research has further identified mutations in key genes in a range of cancers in different organs and tissues (Cieslik and Chinnalyan, 2020). Therefore, the assays developed for the risk assessment of potential carcinogens have been greatly influenced by these observations with the key drivers for classification of carcinogens being the ability to cause mutation and genotoxicity. In assessment of the carcinogenic potential of chemicals, the traditional paradigm considers the reductionist, linear progress from initial chemical exposure to development of a tumour. However, there are issues when applying the approach to certain types of chemicals such as non-genotoxic carcinogens and endocrine disrupters, and when dealing with potential effects from low-dose, long-term exposures.

2. With recent advances in research, COC is now considering a more forward-looking approach which moves away from this simplistic traditional model with a need to reset perceptions of cancer development and the need to incorporate new knowledge from all lines of evidence in our risk assessment methods.

3. Recent research has suggested that although genetic, and epigenetic changes such as hypermethylation, are of great importance in the aetiology of cancer, a number of non-genetic events are also involved (Smith et al, 2016). Hanahan et al. (2000; 2011) further identified these as ‘Hallmarks of Cancer’, namely: genetic instability and mutation; tumour-promoting inflammation; sustained proliferative signalling; insensitivity to anti-growth signals; resistance to cell death; replicative immortality; dysregulated metabolism; angiogenesis; tissue invasion and metastasis; and avoiding immune destruction.

4. Some of these events, such as inflammation, angiogenesis and changes in immune response, suggest that carcinogenesis is influenced by a number of different cell types besides the pre-tumour cells. As a consequence, there has been an increasing level of interest in the potential role the tumour microenvironment may play in the mechanisms by which tumour cells are initiated and develop.

This is a draft position paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced.

The tumour microenvironment

5. Early cancer development is associated with recruitment of a range of different cell types including multipotent stromal cells, epithelial cells, fibroblasts, vascular cells, inflammatory cells and a range of immune cells (including natural killer (NK) and T-cells) in an extracellular matrix, which has become known as *the tumour microenvironment*. These cells produce and regulate a number of growth factors and signals and other cellular modulators (extensively reviewed by Casey et al., 2015).

Cell types and the tumour microenvironment

Blood vessels and vascular endothelium

6. The vasculature around tumour cells is lined by a number of different cell types including fibroblasts, endothelial cells and pericytes. Endothelial cells are activated during tumour progression leading to angiogenesis (an expansion of the vascular network) which supplies the increased oxygen and nutrients needed for tumour expansion (Patan, 2004). Vascular endothelial growth factor (VEGF) is a well-known mitogen which is critical for angiogenesis. A number of chemicals have been shown to affect the expression of VEGF, either in animal models of embryogenesis or lung tumours, including nicotine, oestradiol and the known carcinogen, *N*-nitrosobis(2-hydroxypropyl)amine, although the mechanisms involved are still unclear (reviewed in Casey et al., 2015).

Extracellular Matrix (ECM) and stromal fibroblasts

7. Matrix metalloproteinases (MMPs) appear to be key to the production of the extracellular matrix (ECM) in the tumour microenvironment and are often over-produced in tumour cells (and fibroblasts). Tumour cells undergo a developmental process, epithelial-mesenchymal transition, whereby cells become invasive due to the formation of cell-ECM, rather than cell-cel,l interactions (Orlichenko et al., 2008). Evidence suggests that MMPs are involved in this process. MMPs are regulated by cytokines (e.g. interleukin-1, IL-1), tumour necrosis factor-α (TNF-α), growth factors, bacterial components, hormones and mechanical stress (Shuman Moss et al., 2012).

8. Less is known about the stroma and the effect of changes such as tissue remodelling and chronic inflammation on the development of cancer. However, it does appear that changes in matrix composition and tissue architecture have a major role in cancer development. For example, people with liver cirrhosis are at increased risk of hepatocellular carcinoma and Casey et al. (2015) suggest that most lung cancers develop in people with non-cancer pulmonary disease, including chronic lung disorders such as emphysema and fibrosis.

9. A number of chemical carcinogens are implicated in the tissue remodelling and inflammation which accompanies cancer development, particularly in lung cancer; these include asbestos, tobacco smoke, some heavy metals, crystalline

silica, some forms of radiation and certain organic chemicals. Chemicals may act to remodel tissues by stimulating the release of pro-fibrotic growth factors, cytokine and chemokines to change deposition of collagens and glycoproteins (reviewed in Casey et al., 2015).

Cells of the Immune system and inflammation

10. The role of the immune system in cancer development is complex, involving interactions between the innate immune system and adaptive cells, the production of soluble cytokines and signal factors, and other components of the tumour microenvironment. The innate immune system can both promote and suppress tumourigenesis. Immune cells such as $CD8^+$ T-cells and natural killers (NK) have the ability to kill cancer cells and this may be a natural role for the system.

11. During cancer development, activation of adaptive immune cells may result in tumour cell death, while chronic activation of innate immune cells at sites of pre-neoplastic growth may enhance tumour development (de Visser and Coussens, 2006; Zhang et al., 2018).

12. The importance of the immune response has been recently highlighted by the development of immunotherapy as a major treatment for a number of cancers (reviewed in Rosenberg, 2014). One of the main treatment strategies is based on the presence of inhibitory receptors (e.g. PD-1 and CTLA-4) on the surface of lymphocytes which act as checkpoints on the immune system. These act as targets to be blocked by specific antibodies to ‘release brakes’ and activate the immune response; for example, pembrolizumab (anti-PD-1) and iipilimumab (anti-CTLA-4) are used against a number of cancers including melanoma, renal and non-small cell lung cancers.

13. However, immune responses have also been shown to be suppressed by known carcinogens including the polycyclic aromatic hydrocarbons such as dimethylbenz(a)anthracene (DMBA), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Gallo et al., 1993). A number of hormone-like chemicals have also been shown to modulate the immune response by mechanisms such as macrophage stimulation which produces cytokines. These chemicals include the endocrine disrupters, diethylstilboestrol, bis(2-ethylhexyl)phthalate and p-nonylphenol (Yamashita et al., 2005).

14. Chronic inflammation is associated with an increased risk of cancer formation in many tissues and it has been estimated that 20% of cancer deaths may be influenced by chronic inflammation, including bowel and lung cancer (Balkwill et al., 2001). The mechanisms by which inflammation promotes cancer are complex, but probably involve a number of different cell-types in the tumour microenvironment. Chronic inflammation as a state, attracts activated immune cells, including myeloid-derived suppressor cells and tumour-associated macrophages. These in turn produce a number of cytokines such as interleukins (IL-6, IL-17), interferons (IFNs), Tumour necrosis factor (TNF) and transforming growth factor-beta (TGF-β) and bone

morphogenic proteins (BMPs) which have pro-tumorigenic properties. These proteins activate intracellular signalling pathways (such as NFκB and Wnt) in pre-neoplastic cells thereby promoting proliferation and inhibiting apoptosis (Grivennikov et al., 2010).

15. In addition, chronic inflammation also leads to oxidative stress resulting in the increased production of reactive oxygen species (ROS) which are associated with mutagenic effects. High levels of ROS can cause tissue damage and cell death while lower levels have been linked with increased proliferation. ROS is now thought to have a role as a second messenger and can stimulate the induction of VEGF, and therefore affect angiogenesis, promote cellular proliferation, immune evasion and play a role in cell survival. A number of different cell types in the tumour microenvironment, especially cancer-associated fibroblasts and tumour infiltrating immune cells, are involved in the increased production of ROS. The role of ROS in the tumour microenvironment has recently been reviewed by Weinberg et al. (2019).

16. Overall, the role of the immune system in the microenvironment is complex with potential roles in desirable anti-tumour responses and undesirable pro-tumour chronic inflammatory responses.

The Microbiome

17. The microbiota consists of the commensal bacteria and other microorganisms that colonise the epithelial surfaces of the human body. These have been shown to produce small molecules and metabolites that have both local and systemic effects on cancer development (reviewed by Elinov et al., 2019). Recent research has shown a complex interaction between the microbiota, the immune system and tumours.

18. Some bacterial constituents of the gut microflora are involved in carcinogenesis. For example, *Helicobacter pylori* which causes gastric cancer is an IARC Group 1 carcinogen (IARC 2012). Other examples with a potential association include *Fusobacterium spp.,* with colorectal adenocarcinoma, while patients with colon cancer also have increased numbers of *E. coli.* In mice, it has been shown that the human microbiota may interact with the immune system, for example, through induction of interferon-γ (IFN-γ)- secreting CD^{+}T-cells.

19. The components of the microbiota also have an influence on the responsiveness of chemotherapy treatment, by either increased efficacy or resistance (Elinov et al., 2019). This suggests that chemical carcinogens may also interact with the microbiota.

Present and future methodology

20. There are a number of *in vitro/in vivo* systems available to study the behaviour of the tumour microenvironment during the process of carcinogenesis. Many of these involve the transplantation of neo-plastic cells from different stages in the

development of cancer into a normal tissue microenvironment or, one that is chemically growth restricted.

21. Pre-clinical models of cancer include *in vivo* patient-derived xenografts where human tumours are implanted into mice. There are also *in vitro* models where 3D organ structures are constructed to simulate the pathology of tumours while also attempting to demonstrate the presence of some of the microenvironment. It may be possible for these organoids to be genetically manipulated by CRISPR-Cas technology to include the described mutated genes commonly present in different cancers. In addition, different cell types such as immune, stroma and vascular endothelium can be integrated into these organoids to produce models of the tumour microenvironment.

22. Studies on the human microbiota have often involved use of gnotobiotic (germ-free) mice treated with microbiomes from humans, either healthy, or patients with a defined bacterial mixture. Such techniques have been used for many years however it is at present unclear whether the transplanted microbiota act exactly as those in a normal human environment. Of importance for the study of potential carcinogens, this technique requires that chemicals are metabolised by the mouse, which may not reflect human metabolism. Recent DNA technology could also be used to further “humanise” mice, in attempts to produce better models.

23. Another challenge for research into the role of the microenvironment in tumour development is the integration of information from epidemiological population studies. Recent studies in molecular epidemiology have identified a number of biomarkers of genetic and epigenetic events (such as methylation) and exposure to genotoxic carcinogens (Chen et al., 2005: Vineis and Perera, 2007). Such information is important in the early detection of cancer and the identification of susceptible sub-groups. These studies could be expanded to investigate possible effects of the microenvironment and include biomarkers of immune response, inflammation, vascular signals and markers of histology.

Summary

24. This Position Paper is part of the continuing evolution of a more holistic approach by COC involving collation and consideration of all available evidence in the risk assessment of potential carcinogens. Although such an approach is not yet fully evolved, this paper marks recognition of the importance that the tumour microenvironment may play in carcinogenesis and highlights the need for further research.

25. Mutations in a number of key genes are of vital importance in the development of cancers in many tissues and organs. Therefore, mutational and genetic effects have formed an integral part to date in the assessment of the risks of potential carcinogens. However, it has also been shown that the development of cancer may involve a number of stages that are not driven by mutation alone, but, as outlined above, may include inflammation, angiogenesis, changes in immune

response and tissue invasion. Such processes involve different types of cells which may be present in the microenvironment around the developing clonal cancer cells.

26. Chemicals may affect the cells in the microenvironment and their interactions, and subsequently the development of cancers. The present regulatory methodology for the detection and risk assessment of potential carcinogens (e.g. OECD Test Guidelines) does not adequately consider all the complex mechanisms and interactions in the microenvironment. This being the case, the Committee is actively considering how to undertake a more holistic approach to the risk assessment of potential carcinogens; this includes temporal and spatial aspects that take into account exposure to long-term low-doses of chemicals and also the effects of combined exposure to multiple chemicals.

COC Position Paper 2020/TBC

Date TBC

This is a draft position paper for discussion.
It does not reflect the view of the Committee and must not be quoted, cited or reproduced.

Abbreviations

BMP - bone morphogenic proteins

CTLA-4 – cytotoxic T-lymphocyte-associated protein 4

DMBA - dimethylbenz(a)anthracene

ECM - extracellular matrix

IFN - interferon

MMP - Matrix metalloproteinase

NK - natural killer cells

NNK - 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

PD-1 – Programmed cell death protein 1

ROS - reactive oxygen species

TGF-β - transforming growth factor-beta

TNF - tumour necrosis factor

VEGF - vascular endothelial growth factor

References

Balkwill, F. and Mantovani, A. (2001) Inflammation and cancer: back to Virchow? Lancet, 357, 539-545.

Boveri, T. (2008) Concerning the origin of malignant tumours. Translated annotated by Henry Harris. J. Cell Science, 121, 1-84.

Casey, S.C., Vaccari, M., Al-Mulla, F., Al-Temaimi, R., Amedei, A. and 30 others (2015) The effect of environmental chemicals on the tumor microenvironment. Carcinogenesis, 36, Suppl. 1, S160-183.

Chen, Y-C. and Hunter, D.J. (2005) Molecular epidemiology of cancer. CA Cancer J. Clin. 55, 45-54.

Cieslik, M. and Chinnalyan, A. (2020) Global cancer genomics project comes to fruition. Nature, 578, 39-40.

deVisser, K.E., and Coussens, L.M. (2006) The inflammatory microenvironment and its input on cancer development. Contrib. Microbiol. 13, 118-137.

Elinov, E., Garrett, W.S., Trinchieri, G. and Wargo, J. (2019) The cancer microbiome. Nature Reviews Cancer, 19, 371-376.

Gallo, F., Morale, M.C., Sambataro, D., Farinella, Z., Scapagnini, U. and Marchetti, B. (1993) The immune system response during development and progression of carcinogen-induced rat mammary tumors: prevention of tumor growth and restoration of immune responsiveness by thymopentin. Breast Cancer Res. Tr. 27, 221-237.

Goodson, W.H., Lowe, L., Carpenter, D.O. et al. (2015) Assessing the carcinogenic potential of low-dose exposure to chemical mixtures in the environment. Carcinogenesis, 36, Suppl. 1 S254-S296.

Grivennikov, S.I., Greten, F.R. and Karin, M. (2010) Immunity, inflammation, and cancer. Cell, 140, 883-899.

Hanahan, D. and Weinberg, R.A (2000) The hallmarks of cancer. Cell, 100, 57-70

Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. Cell, 144, 646-674.

IARC (2012) A review of human carcinogens, Biological Agents Vol. 100B. IARC Monographs on the Evaluation of carcinogenic risks to humans.

Orlichenko, L.S. and Radisky, D.C. (2008) Matrix metalloproteinases stimulate epithelial-mesenchymal transition during tumor development. Clin. Exp. Metastasis, 25, 593-600.

Patan, S. (2004) Vasculogenesis and angiogenesis. Cancer Treat. Res., 117, 3-32.

Rosenberg, S.A. (2014) Decade in review – Cancer immunotherapy. Enering the mainstream of cancer treatment. Nat. Rev. Clin. Oncol. 11, 630-632.

Shuman Moss, L.A., Jensen-Taubman, S. and Stetler, W.G. (2012) Matrix metalloproteinases: changing roles in tumor progression and metastasis. Am. J. Pathol., 181, 1895-1899.

Smith, M.T., Guyton, K.Z., Gibbons, C.F., Fritz, J.M., Portier, C.J., Rusyn, I.,

DeMarini, D.M., Caldwell, J.C., Kavlock, R.J., Lambert, P., Hecht, S.S., Bucher, J.R., Stewart, B.W., Baan, R., Cogliani, V.J. and Straif, K. (2016) Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis. Environ Health Perspect., 124, 713-721.

Vineis P. and Perera, F. (2007) Molecular epidemiology and biomarkers in etiologic cancer research: the new in search of the old. Cancer Epidemiol. Biomarkers Prev., 16, 1954-1965.

Weinberg, F., Ramnath, N. and Nagrath, D. (2019) Reactive oxygen species in the tumor microenvironment. Cancers (Basel) 11, 1191. Published online doi:

10.3390/cancers11080191.

Yamashita, U., Sugiura, T., Yoshida, Y. and Kuroda, E. (2005) Effect of endocrine disrupters on macrophage function in vitro. J. UOEH, 27, 1-10.

Zhang, S., Yang, X., Wang, L. and Zhang, C. (2018) Interplay between inflammatory tumour microenvironment and cancer stem cells (Review). Oncology Lett.16, 679686.