厚生労働行政推進調査事業費補助金（化学物質リスク研究事業）

OECDプロジェクトでの成果物を厚生労働行政に反映させるための研究

令和4年度 分担研究報告書

発がん性試験のIATA及びAOP開発

研究分担者　小川久美子　国立医薬品食品衛生研究所病理部　部長
研究協力者　西川秋佳　　国立医薬品食品衛生研究所病理部　客員研究員

研究要旨

2016 年に経済協力開発機構 (OECD: Organisation for Economic Co-operation and Development) において、 非遺伝毒性発がん物質の統合的評価手法の確立を目標とした integrated approach to the testing and assessment (IATA) of non-genotoxic carcinogens (NGTxC) の専門家グループが立ち上げられた。非遺伝毒性発がん性の機序に関連する事象を抽出し、それぞれの事象を主に *in vitro* の試験系で評価する方法とその妥当性について、議論が継続されている。本研究では、当該 IATA 開発に協力すると共に、発がん性の有害転帰経路（Adverse Outcome Pathway: AOP）の開発、並びに生体における発がん機序に関する調査研究を実施し、化学物質のヒト発がん性に関する適切な評価を推進し、以て、日本の厚生労働行政に資することを目的としている。

げっ歯類における化学物質誘発鼻腔発がんの網羅的解析結果からは、各種鼻腔腫瘍の前駆病変は、遺伝毒性の有無に関係なく、腫瘍発生部位における化学物質誘発性の細胞毒性と関連している可能性があると考えられた。また、分子開始イベント後の経路は、遺伝毒性発がん物質と非遺伝毒性発がん物質の間で大きく重複している可能性が示唆された。

OECD 専門家グループの cell proliferation のサブグループにおいては、細胞増殖の評価に関する *in vitro* / short term *in vivo* アッセイの適応・留意点・限界などを内容とする論文ドラフト案についてメール及び web 会議にて議論した。細胞増殖の評価では、*in vitro* アッセイのみならず、短期や慢性の *in vivo* 毒性試験における、バイオマーカーを用いた詳細な検討も有用であることが議論された。

2023 年 2 月 17 日には、各ブロックの進捗状況について、web 会議にて情報共有を行い、全体の取り纏めが図られた。IATA 開発においては、新規アッセイ法の有用性について注視すると共に、アッセイ系の評価が適切になされるよう、引き続き協力を続ける必要があると考えられた。

A. 研究目的

2016 年に経済協力開発機構 (OECD: Organisation for Economic Co-operation and Development) において、非遺伝毒性発がん物質の統合的評価手法の確立を目標とした integrated approach to the testing and assessment (IATA) of non-genotoxic carcinogens (NGTxC) の専門家グループが立ち上げられた。非遺伝毒性発がん性の機序に関連する事象を抽出し、それぞれの事

象を主に *in vitro* の試験系で評価する方法とその妥当性について、議論が継続されている。本研究では、当該 IATA 開発に協力すると共に、発がん性の有害転帰経路（Adverse Outcome Pathway: AOP）の開発、並びに生体における発がん機序に関する調査研究を実施し、化学物質のヒト発がん性に関する適切な評価を推進し、以て、日本の厚生労働行政に資することを目的としている。

B. 研究方法

B-1. 発がん性の AOP 開発

研究協力者西川が主体となり、ホルムアルデヒド誘発鼻腔発がん機序に関する論文に引き続き、各種化学物質暴露による鼻腔発がん全般の AOP に関する論文作成を実施した。ラット、マウス、ハムスターに鼻腔腫瘍を誘発する化学物質について、PubMed の文献に加えて、NTP、IARC、日本バイオアッセイ研究センターのデータベースを使用して情報収集した。誘発された鼻腔腫瘍について、動物種、投与経路、組織型を分類し、更には、関連する非腫瘍性病変及び遺伝毒性のデータについても抽出し、腫瘍発生経路の推定を取り纏め、投稿した。

B-2. 非遺伝毒性発がん性の IATA 開発への協力

非遺伝毒性発がん性IATA開発専門家のweb会議に参加し、開発方針に関する議論及び最新の評価方法に関するwebinarに参加した。全体会合に加えて、当該IATAにおける13のアッセイブロックの内2つまたは3つを分担し、そのサブグループ会議にも参加し、候補となるアッセイの利用に関する考え方など論文化について議論した。

（倫理面への配慮）

該当なし

C. 研究結果

C-1. 発がん性の AOP 開発

網羅的に情報収集した鼻腔発がん物質のうち 40 種の吸入暴露による発がん物質（ラット 38 物質、マウス 11 物質、ハムスター5 物質）及び 38 種の非吸入暴露による発がん物質（ラット 36 物質、マウス 5 物質、ハムスター17 物質）について誘発された鼻腔腫瘍を、国際統一毒性病理用語・診断基準 (International Harmonization of Nomenclature and Diagnostic Criteria: INHAND)に基づいて分類した結果、扁平上乳頭腫、扁平上皮癌、腺腫、腺癌、腺扁平上皮癌、神経上皮癌、未分化癌、非特異的な癌、線維肉腫、血管腫、血管肉腫、粘表皮腫、横紋筋腫、横紋筋肉腫が報告されていた。最も高頻度の鼻腔腫瘍は扁平上皮癌であり、投与経路に関係なく認められ、その前駆病変として、扁平上皮化生および/または扁平上皮乳頭腫と呼吸上皮過形成が示唆された。 2 番目に多いのは腺癌であり、その前駆病変として主に嗅上皮過形成が示唆されたが、腺腫の前駆病変は呼吸上皮病変と考えられた。これらの経路はげっ歯類間で共通していると考えられるが、マウスまたはハムスターのデータは限定的であった。本内容について、論文化を進めた。

C-2. 非遺伝毒性発がん性の IATA 作成への協力

OECD で進められている非遺伝毒性発がん性の IATA 開発に協力した。2018 年 6 月の会議において、非遺伝毒性発がん性に係る試験・検査のパラメータを優先順位に関して 4 つのカテゴリーに分けることになった。また、候補となるアッセイを 13 のブロ

ックに分けて、分担してレビューしている。

IATA 開発について、小川研究分担者は cell proliferation 及び resistance to apoptotic cell death のサブグループに、西川研究協力者は cell transformation, indicator of oxidative stress 及び resistance to apoptosis cell death のサブグループに参画し、アッセイブロックの評価を行った。

Cell proliferation のサブグループにおいては、細胞増殖の評価に関する *in vitro* / short term *in vivo* アッセイの適応・留意点・限界などを内容とする論文ドラフト案についてメール及び web 会議にて議論した。小川研究分担者は、*in vivo* アッセイに関するセクションを担当し草案を作成した。

2023 年 2 月 17 日には、各ブロックの進捗状況について、web 会議にて情報共有を行い、全体の取り纏めが図られた。

添付資料

Draft summary record of the teleconference of the expert group on the development of an IATA for non-genotoxic carcinogens (NGTxC)

D. 考察

D-1. 発がん性の AOP 開発

げっ歯類における化学物質誘発鼻腔発がんの網羅的解析結果から、各種鼻腔腫瘍の前駆病変は、遺伝毒性の有無に関係なく、腫瘍発生部位における化学物質誘発性の細胞毒性と関連している可能性があると考えられた。また、分子開始イベント後の経路は、遺伝毒性発がん物質と非遺伝毒性発がん物質の間で大きく重複している可能性が示唆された。

D-2. 非遺伝毒性発がん性の IATA 開発への協力

OECDの非遺伝毒性発がん性のIATA開発において、特に細胞増殖の評価では、*in vitro* アッセイのみならず、短期や慢性の*in vivo* 毒性試験における、バイオマーカーを用いた詳細な検討も有用であることが議論された。今後とも、新規アッセイ法の有用性について注視すると共に、アッセイ系の評価が適切になされるよう、引き続き協力を続ける必要があると考えられた。

E. 結論

E-1. 発がん性の AOP 開発

げっ歯類における化学物質誘発鼻腔発がんの網羅的解析は、化学物質によって誘発される鼻腔腫瘍の病因の包括的な理解に貢献し、細胞毒性から鼻腔腫瘍発生に至るAOPの解明、および非遺伝毒性発がん物質のIATA開発などのOECDの活動に資するものと考えられた。

E-2. 非遺伝毒性発がん性の IATA 開発への協力

OECD の非遺伝毒性発がん性の IATA 開発の議論には、引き続き協力していく必要があると考えられた。

F. 研究発表

F-1. 論文発表

該当なし

F-2 学会発表

1. 小川久美子、長野嘉介、小島 肇、福島昭治、西川秋佳. 鼻腔発がんの機序について−AOP的考え方. 第5回医薬品毒性機序研究会. 令和4年12月9日、東京.
2. 小川久美子.「OECDテストガイドライン」と「バイオアッセイ」. 安全性評価技術 “Bhas42CTA”の「医薬品、化学物質、電磁

場への社会実装」神奈川県・横浜国立大学 共同研究講座シンポジウム. 令和5年2月17日、横浜.

G. 知的財産権の出願・登録状況

1. 特許取得

該当なし

2. 実用新案登録

該当なし

3. その他

該当なし

Draft summary record of the teleconference of the expert group on the development of an IATA for non-genotoxic carcinogens (NGTxC)

17 February 2023

Participants

Martin Paparella (Austria); Daniel Desaulniers (Canada); Pavel Babica (Czech Republic); Jochen vom Brocke (EC/ECHA); Kimmo Louekari (Finland); Marc Audebert, Paule Vasseur (France); Annamaria Colacci, Monica Vaccari (Italy); Kumiko Ogawa, Kiyomi Ohmori (Japan); Betty Hakkert, Mirjam Luijten (Netherlands); Maria Dusinska (Norway); Miriam Jacobs (UK); Paul Brown, Danica E. DeGroot, Suzanne Fitzpatrick, Abby Jacobs, Tim McGovern (US); Marco Corvaro, Maria Donner, Christian Strupp, (BIAC); Gina Hilton, Donna Macmillan (ICAPO); Yusuke Oku (OECD Secretariat),

Invited experts: Iris Carrico (EC/JRC)

Introduction only: Raffaella Corvi (EC/JRC); Tomoko Aoyagi, Patience Browne, Nathalie Delrue (OECD Secretariat)

Objectives

The purpose of the teleconference was:

- To present the status of the work on the assay blocks with respect to the manuscripts close to publication for the Special Issue in the International Journal of Molecular Sciences (IJMS)
- To initiate the discussion on the regulatory framework of the NGTxCs IATA
- To agree on next steps.

Item 1. Introduction

- **Updates from the Secretariat**

The TC was opened by the Secretariat and updates provided on related activities. Nathalie Delrue (OECD) informed the group that the development of an IATA for NGTxC was highlighted as a priority at the last meeting of the Chemicals and Biotechnology Committee (i.e. the Committee overseeing the Chemical Safety Programme at the OECD). Tomoko Aoyagi (OECD) introduced the IATA Case Study Project and the current call for carcinogenicity IATA Case Studies. Following a pilot Case Study on carcinogenicity of agrochemicals (submitted by ICAPO/ReCAAP Consortium), the OECD is now calling for more Case Studies on Carcinogenicity for evaluating human carcinogenicity using a variety of approaches and in different chemical sectors. In this context, the Expert group was invited to submit the draft NGTxC IATA when ready to the IATA Case Study programme (CSP). Cooperation between Test Guidelines Programme and the IATA CSP is important and offers the opportunity to receive feedback on the draft NGTxC IATA from the IATA CSP before submission to the WNT. In addition, expert group members were invited to consult their networks and consider the submission of IATA Case Studies on human carcinogenicity within the next review cycles of the OECD Case Study Project.

- **Project progress**

Miriam Jacobs, chair/project lead (UK) provided an overview of the NGTxC IATA development project. The work was accepted, as a project on the OECD TGP workplan in 2016, with the identification of key mechanisms and promising assays for inclusion in the IATA. A framework where these assays would fit was designed, with the view to encompassing different theories on cancer and being applicable across sectors and jurisdictions. Several papers have been published in the literature, including a thought starter and an uncertainty analysis of the Rodent Cancer Bioassay. In 2018, the EG agreed on a general AOP like structured NGTxC IATA, including assay blocks, based on hallmarks of cancer. The EG then started conducting critical reviews of assays in the various blocks identified. Assessment criteria were developed tested and revised. Many of the reviews include the assays' appropriateness and readiness to enter the Test Guidelines Programme.
It was indicated that most of the subgroup reviews have been completed now and that the remaining ones will be completed this year (see also item 7).
Several AOPs developed/finalised in the AOP-Wiki can also be used and support the work. Examples were given in the presentation. The publication and the slides presented are available in the community site on Expertise in TG development.

The table below lists the various assay blocks identified for the IATA and the corresponding publications under way or completed (See the list of publication in the Annex).

Key hallmarks identified in the IATA	**Manuscripts underway or completed in IJMS Special Issue or in other journals**
Receptor binding CYP P450 induction	Receptor mediated CYP induction (publication in Front.Reg.Tox)
Cell proliferation*	Cell proliferation (Strupp *et al.*, in preparation)
Cell transformation*	CTA transcriptomics (2 papers) CTA historical review (Colacci *et al.,* submitted[1])
Gap junction	Gap junction
Indicators of oxidative stress	
Immunosuppression and immunoevasion	Immunosuppression/evasion (in preparation, building upon the immunotoxicity DRP– will be presented at Eurotox 2023)
Gene expression and cell signalling pathways*	Gene and cell signalling (Oku et al. 2022)
Apoptotic cell death (manuscript in preparation)	Apoptosis (in prep)
Pathogenic angiogenesis and neoangiogenesis	deprioritised
Genetic instability	
Cellular senescence	Senescence (in prep)
Metastasis	deprioritised
Epigenetics mechanisms	Epigenetics (Desaulniers et al 2020)
Others	Computational approach from Lhasa (publication in ALTEX)
	Regulatory framework (in prep for special Issue 2023)

* **In bold**: Presented/discussed at the TC

[1] Now published : https://www.mdpi.com/1422-0067/24/6/5659

Item 2. Presentation of the paper on Cell signalling and transcriptomics (Yusuke Oku - Secretariat, Miriam Jacobs - UK, Federica Madia – JRC, Tim Mc Govern, US FDA)

Yusuke Oku (OECD) presented the review paper published in the IJMS last year on cell signaling and transcriptomics. The publication and the slides presented are available in the community site on Expertise in TG development [Link]. The authors have reviewed assays in Block 7 and examined how the omics tools and resources that are currently available, can be utilised to pre-screen the changes in cell signalling pathways possibly leading to Key Event (KE) precursor steps of the carcinogenic phenotypic outcome. The group proposed that the whole transcriptomics combined with pathway analyses such as Gene Set Enrichment Analysis (GSEA) can be used as a pre-screening tool of the biological assays for each biological process. The manuscript also describes elements to be considered for the regulatory use of transcriptomics for pre-screening in the IATA, e.g., the use of relevant cell lines, the duration of exposure and concentration.

Item 3. Update on Cell proliferation paper (Christian Strupp, Marco Corvaro - BIAC, Kumiko Ogawa-Japan)

Christian Strupp (BIAC) presented the manuscript in preparation on cell proliferation, for submission in IJMS. The manuscript provides the outcome of the cell proliferation assay review by the subgroup, as discussed over the last years, with the participation of additional experts in the field. A ranking of the various types of assays is proposed in relation to their readiness for future development and validation i.e. A for proliferation markers (in vivo), B for in vitro proliferation in primary cells and C for assays addressing only cell number. In addition, it addresses some common misunderstanding related to cell proliferation.

The technical part of the manuscript is now completed; the authors now are seeking guidance and input from the whole group on how to integrate these assays in the IATA. It was suggested that the paper could include basic recommendations the expert group could build upon at a later stage when considering the various manuscripts. Basic recommendations have been provided by the chair and ECHA colleagues, and include the use of proliferation markers from acute and chronic in vivo studies as part of exiting information, and in the MIE and early to mid KEs of the IATA.
Specifically in relation to (i) screening level and identification of the target organ and (ii) more definitive mechanistic studies, i.e., to confirm a Mode of action hypothesis. The authors will continue to work on the manuscript, finalise and submit it.

Miriam Jacobs (UK) informed the group that a figure mapping of the different recommended tests, their readiness and where they could fit in the IATA is in preparation and would support the next steps, i.e. building the IATA.

Item 4. Update on Cell transformation assay historical review paper (Annamaria Colacci - Italy, Raffaella Corvi-JRC, Paule Vasseur, Kiyomi Ohmori) – (different from evaluation of CTA)

Annamaria Colacci (Italy) presented a historical review of the Cell Transformation Assay (CTAs) and how mechanistic insight has been gained in the recent years, which could facilitate the uptake of the CTAs in the future IATA for NGTxC. The work was recently submitted for publication in IJMS. It was noted that this is different from the subgroup review of CTAs, which was conducted separately. It was explained that the main reason the CTAs were not adopted as Test Guidelines at the OECD 10 years ago was the lack of mechanistic understanding at that time. During the period that followed, what might be called 'the omics era of CTAs', omics have been used to better understand CTA mechanism, highlighting the role of immune-mediated inflammation pathways towards onco-transformation.

Further work still needs to be done to improve the reproducibility of CTAs and to reproduce the work that has been done on omics. Miriam Jacobs (UK) suggested that there could be opportunities in PARC for more validation, or under auspices of the OECD via the submission of project proposals (SPSFs). Resources will be needed and it was mentioned that the OECD recently sent an open call to grant-making institutions in member countries for an urgent mobilisation of national and regional resources to support validation. This will hopefully encourage funders to support future CTA validation related initiatives.

Item 5. NGTxC IATA draft Regulatory framework – initial considerations and plans

Kimmo Louekari (Finland) presented a preliminary proposal for a Modular testing strategy (MOST) for the testing and assessment of NGTxC. It is organised in modules and was developed with the view to be flexible enough but not too flexible.

The structure of the modules is the following:

- Module A – Available information (Existing (regulatory) data, e.g. endocrine activity screening, systematic toxicity, carcinogenic literature, omics, structural and read across information, QSARs, exposure)
- Module B – Initial Key Event (MIE) and metabolism
- Module C – Mechanistic assays (inflammation, immune response, mitotic signalling, cell injury (gap junction inhibition))
- Module D – Cell proliferation and cell transformation
- Module E – Late adverse outcome, non-adaptative, non-transient
- Module F – Conclusion through weighing the evidence

It was noted that it would be useful to draw a conclusion at the end of each module. We don't know under which legislation the IATA GD will be used when approved but as an example, conclusion of Module C could include several scenarios, e.g.:
Scenario 1: If no evidence on any on the 4 endpoints of module C, and no other indication of toxicity from previous modules → modular strategy stopped and no need for further testing
Scenario 2: Some of the studies are positive → weight of evidence recommended based on the module C assay results and other information available
Scenario 3: Contradicting data from Module C → run the weight of evidence analysis and figure out if further testing / prioritisation for further work is needed.

The presentation was well received. Further discussion is needed by the EG and it was thus proposed to organise a dedicated TC in the near future on the regulatory framework. It can be organized as soon as the other pieces discussed previously are consolidated e.g., cell proliferation; subsequent steps the cell transformation can address etc. Other aspects will be considered too such as new work on ICH (S1B), ReCAAP.

Item 6 – International perspectives – participants will be invited to present other related international initiatives for information and cooperation purposes, e.g. PARC in Europe

Daniel Desaulniers (Canada) reported that his group recently published an experimental paper on the DNA methylation changes in Syrian hamster fetal cell transformation by benzo[a]pyrene (Desaulniers D et al. 2023 Toxicology).

Item 7. Overarching discussion and planning for next steps

The EG was informed of upcoming webinars (in February – March - display available) organised by PCRM/ICAPO on New Approach Methodology (NAM) Use for Regulatory Application (NURA).
In addition, the following opportunities to meet were proposed:

- Webinar like teleconferences through the year to discuss relevant topics such as:
  - Developed AOPs of interest, e.g., AOP on breast cancer
  - New experimental work on SHE (see item 6)
  - EPAA work
- TC on project proposals for PARC in view of a deadline in May 2023 (proposals for projects to start next year)
- **TC on the development of regulatory framework**, plus considerations of the evolution in various regulatory jurisdictions (to be organised shortly)
- Satellite meeting at the Eurotox meeting (September 2023): discussion of immunoevasion – opportunity for those who will join to meet in person
- **OECD face to face**, if we are ready in Autumn 2023.

There was support for these meeting options. Regarding the face-to-face meeting, the primary objective of it will be to discuss with the entire group the outcome of the subgroup assay reviews that were initiated following the EG meeting in 2018 and integration into the IATA. This would include the reviews that have been subject to the development of a manuscript.

Early summer, in advance of the meeting, a document will be prepared compiling all the evaluations conducted so far. This document will be circulated and will serve as a basis for the discussion of the EG on assay priorities. It will also help identifying potential gaps, making sure all the assays within a block have been considered (it was noted for example that for the oxidative stress, the ToxTracker is not the only assay and furthermore it is developed primarily to provide mechanistic insight for genotoxicants, not for NGTxC, however there are aspects of cross-over).

In addition to the assay evaluation, other objectives of the face-to-face meeting would also be to (i) consolidate a pragmatic IATA, (ii) agree on what sort of case studies could be initiated. It was noted that assay priority setting should be considered and assays that are close to validation should be proposed for OECD TGs development via the preparation of SPSFs at an appropriate time. The draft Guidance Document on the IATA should be initiated in parallel to the proposals for TG development since both activities would support each other.

Annex: Links to published IJMS Special Issue papers

Sovadinová, I.; Upham, B.L.; Trosko, J.E.; Babica, P. Applicability of Scrape Loading-Dye Transfer Assay for Non-Genotoxic Carcinogen Testing. Int. J. Mol. Sci. 2021, 22(16), 8977; https://www.mdpi.com/1422-0067/22/16/8977

Desaulniers, D.; Vasseur, P.; Jacobs, A.; Aguila, M.C.; Ertych, N.; Jacobs, M.N. Integration of Epigenetic Mechanisms into Non-Genotoxic Carcinogenicity Hazard Assessment: Focus on DNA Methylation and Histone Modifications. Int. J. Mol. Sci. 2021, 22(20), 10969; https://www.mdpi.com/1422-0067/22/20/10969

Ohmori, K.; Kamei, A.; Watanabe, Y.; Abe, K. Gene Expression over Time during Cell Transformation Due to Non-Genotoxic Carcinogen Treatment of Bhas 42 Cells. Int. J. Mol. Sci. 2022, 23(6), 3216; https://www.mdpi.com/1422-0067/23/6/3216

Pillo, G.; Mascolo, M.G.; Zanzi, C.; Rotondo, F.; Serra, S.; Bortone, F.; Grilli, S.; Vaccari, M.; Jacobs, M.N.; Colacci, A. Mechanistic Interrogation of Cell Transformation In Vitro: The Transformics Assay as an Exemplar of Oncotransformation. Int. J. Mol. Sci. 2022, 23(14), 7603; https://www.mdpi.com/1422-0067/23/14/7603

Oku, Y.; Madia, F.; Lau, P.; Paparella, M.; McGovern, T.; Luijten, M.; Jacobs, M.N. Analyses of Transcriptomics Cell Signalling for Pre-Screening Applications in the Integrated Approach for Testing and Assessment of Non-Genotoxic Carcinogens. Int. J. Mol. Sci. 2022, 23(21), 12718; https://www.mdpi.com/1422-0067/23/21/12718

Colacci, A.; Corvi, R.; Ohmori, K.; Paparella, M.; Serra, S.; Da Rocha Carrico, I.; Vasseur, P.; Jacobs, M.N. The Cell Transformation Assay: A Historical Assessment of Current Knowledge of Applications in an Integrated Approach to Testing and Assessment for Non-Genotoxic Carcinogens. Int. J. Mol. Sci. 2023, 24(6), 5659; https://www.mdpi.com/1422-0067/24/6/5659