Bundesamt
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Spotlight on EMF Research

Spotlight on “Genetic profiling of rat gliomas and cardiac schwannomas from life-time radiofrequency radiation exposure study using a targeted next-generation sequencing gene panel” by Brooks et al. in PLoS One (2024)

Category [radiofrequency, animal study]

Spotlight - Jun/2024 no.2 (Eng)

Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

The National Toxicology Program (NTP) and the Ramazzini Institute (RI, to which the authors of the manuscript at hand are affiliated) have previously performed studies on health effects of lifetime exposure of rats to radiofrequency electromagnetic fields (RF-EMF). Although these studies are not entirely consistent, they indicate an increased incidence of malignant gliomas (a common type of brain tumour) and malignant schwannomas of the heart, especially in RF-EMF-exposed male rats. The studies have some limitations, which have been reported in comments by the BfS [2-4]. Furthermore, recent more informative epidemiological studies did not find an increased risk for brain cancer in humans due to RF-EMF exposure [5, 6]. Nevertheless, it remains unclear whether the gliomagenesis that might have occurred under the specific circumstances of the rat studies has any translational relevance for human tumours.

2 Results and conclusions from the authors‘ perspective

Brooks et al. [1] aimed to characterise the genetic alterations in the malignant rat tumours obtained during the RI study to assess their significance for human disease. Rats had been either sham-exposed or exposed to far field RF-EMF at 0.001, 0.03 or 0.1 W/kg whole-body specific absorption rate (SAR). Brooks et al. retrieved archive specimens from 14 gliomas and nine heart schwannomas in total, including two brain and one heart tumour that spontaneously formed in control rats. All tumours were from two-year old rats. If RF-EMF exposure causes mutations, the authors expected these events to be already detectable in adult rats, before the tumours manifested at the age of two years. Therefore, thirty RF-EMF-exposed non-tumour (ENT) rat brain tissues were taken at a one-year interim time point. Furthermore, age-matched normal

tissues from nine unexposed rats each were used as controls. Kidney tissue DNA was available from each rat to control for germline variants.

Histopathological characterisation of the tumours revealed that malignant rat gliomas resembled low-grade diffuse human gliomas.

A targeted next-generation sequencing (NGS) panel of 23 genes most relevant to human brain tumours was designed to include either the full protein-coding sequence of the genes, or specific 'hotspot' positions that are frequently and distinctly mutated in human gliomas. The generated DNA libraries covered a total of 64,759 base pairs. Libraries from all the tumours and normal tissues were sequenced at a coverage of >1000x. Two bioinformatic methods were used to process the sequencing data, align it to the rat Rn6 reference genome, and detect variants at allele frequencies of >1%. Variants detected in kidney DNA that differed from the known rat genome sequence were filtered out from the tissues of interest. Similarly, identified alterations common to both RF-EMF-exposed and unexposed rat samples were excluded. After filtering, the authors focused on the remaining mutations found in RF-EMF-exposed tumour and non-tumour tissues.

A multitude of exonic point mutations, i.e. affecting the protein coding sequence, were found: 1058 in gliomas, 397 in exposed normal brain tissue (ENT) and 744 in schwannomas. Gliomas and ENT shared 38 alterations.

Those mutations that were predicted to be deleterious to the function of the encoded protein were compared with all documented mutations in all cancer types available in the Catalogue of Somatic Mutations in Cancer (COSMIC) database. About 25% of the specific alterations identified in this study are also reported in COSMIC. The relevance of specific mutations to human cancers is variable, with some genes (*Tp53*, *Cdkn2a*, *Erbb2*, *Chek2*, *Kras* and *Pik3r1*) harbouring many alterations matching COSMIC entries while the opposite was true for other genes (*Idh1/2*, *Atrx*, *Notch1*, *Pten*, *Rb1* and *Setd2*). In contrast to human gliomas, which often have hotspot mutations in the *IDH1* and *IDH2* genes, no corresponding mutations were found in the rat tumours. Although *Setd2* was found altered in every glioma and cardiac schwannoma in this study, only one nonsense mutation is reported in COSMIC.

Brooks et al. performed the first reported targeted NGS analysis of genetic alterations in rat gliomas. The authors conclude from their results that either partially different gene mutations than in humans are important for the development of cancer in rats, or that HF-EMF causes a different spectrum of mutations than the one observed in human gliomas. In contrast to most human gliomas, rat gliomas carry *Idh1*/*Idh2* wild-type genes, regardless of whether the tumours developed spontaneously or due to exposure to RF-EMF. In addition, some of the genetic alterations detected in gliomas were also found in exposed normal brain tissue, providing insights into the molecular pathogenesis during lifelong exposure to RF-EMF.

3 Comments by the BfS

Although RF-EMF radiation has been claimed to cause genetic damage [7], no studies have clearly and reproducibly revealed the manifest consequences of such lesions at the DNA level in terms of somatic mutations in normal or tumour tissues. Brooks et al. analysed mutations in 23 human cancer-related genes in tumours arising in rats from a previous study on lifetime exposure to RF-EMF [8]. Gliomas rarely occur in rats, and even carcinogenicity studies in rats only occasionally observe them at increased incidences. Accordingly, only few data exist on the molecular genetic status of rat gliomas [1]. The present study is the first to carry out a detailed histopathological examination and a molecular genetic NGS analysis of such tumours. A noteworthy finding of the study is that rat gliomas lack mutations in *IDH1* and *IDH2* which frequently occur in human gliomas. Consequently, these rat tumours most closely resemble human low-grade IDH wild-type gliomas. According to the authors, however, the small number of gliomas and schwannomas available does not allow drawing reliable conclusions about mutational patterns. On that note, the study objectives are not very plausibly formulated. Finding translational relevance between rats and humans with regard to the molecular characteristics of their gliomas and cardiac schwannomas, as

intended by the authors, would be of limited benefit from the outset. This is because gliomas rarely occur in rats, even in the few carcinogenicity studies associated with glioma incidence. Thus, an unjustifiably high number of rats would have to be used in animal experiments for the rat to serve as a model to study glioma in the future. On the other hand, cardiac schwannomas are also rare in rats and extremely rare in humans [9]. A meaningful objective, but only marginally stated by the authors, was to provide evidence that mutations could be caused by RF-EMF exposure, in particular by utilizing the ENT samples. However, as explained below, the approach to this question lacks the necessary scientific rigour.

The paper contains some contradictions in text and figures which impede the readability of the paper and cast doubt on its reliability: For instance, the data on all detected single nucleotide variants in ENT, as shown in the corresponding figure, do not agree with other elements of the paper related to this data. It is therefore not entirely clear which data on ENT are correct.

Further limitations impede the interpretability of the findings. The mutation profiles of spontaneous tumours are not indicated in the figures or discussed in the text. Although a total of only three tumours from unexposed rats were analysed, their data could still have revealed whether there were fundamental differences that set them apart from the tumours allegedly caused by RF-EMF exposure. The initial publication by the RI did not mention rats that were to be sacrificed for analysis at an interim time point [8] as part of the study design. Whether the ENT material was obtained under the very same experimental conditions as samples from the lifetime exposure cohorts is therefore not entirely clear. Furthermore, the authors state that custom filters were used to call mutations for each sample separately, making it difficult to compare the different samples directly. The allele frequencies for each mutation were not provided. There was also no mention of how exactly mutations were defined as deleterious and how many deleterious mutations overlapped between gliomas and ENT. Furthermore, frequently detected mutations could have been validated by other methods, e.g. Sanger sequencing.

The study assesses the relevance of the detected mutations by searching the COSMIC database for analogous mutations reported in human cancers. It would have been more conclusive to directly compare the mutation profiles with those of human diffuse low-grade gliomas. Examining data from the GLASS consortium [10], some frequently mutated genes in *IDH* wild-type human glioma are characterized by deletions or amplification. Therefore, the authors could have added copy number alteration analysis for the relevant genes. The comparison with human gliomas also reveals that mutations in some genes are mutually exclusive in the human tumours. In contrast, many rat gliomas and schwannomas in this study contained mutations in the majority of the 23 genes co-occurring in the same tumour, including genes in which mutations are most often mutually exclusive. The authors did not address this conspicuity. In addition, nonsense mutations, which are likely to disable protein function, were frequently detected in oncogenic genes such as *Egfr*. This is unexpected because tumour-promoting mutations in these genes usually result in hyperactivity of the protein products. These contradictions reduce the interpretability of the results found in this study.

The biggest flaw in the paper is that the mutation profiles of age-matched unexposed normal tissues were not reported. Mutations that overlapped between unexposed and exposed tissues were excluded as false positives without further information, and the data from unexposed normal tissues were not considered further. Hence, it remained unclear whether, which and how many base changes were present in the unexposed normal tissues after exclusion of the overlap. A similar total number of mutations in unexposed tissues, but at different base positions than in tumours and ENT, would indicate an age-related increase in mutational load, as shown by Li et al. [11] for aged human tissue. This would argue against a mutagenic effect of RF-EMF exposure, as implied by the authors. A further limitation and a possible reason why the analysis of unexposed normal tissues was not presented by the authors despite the availability of the data, are the highly heterogeneous sequencing depths between the tissues of interest, which varied up to a factor of 500. It was not shown whether the read depth differed on average between RF-EMF-exposed and unexposed tissues. A high depth in one group of samples would allow the confident detection of rare variants, while the same variants would not score in another group of samples sequenced at much lower

depths. Thus, the total number of called mutations would be confounded if the sequencing depths were unevenly distributed between groups.

The present study takes an important step towards creating a database on potential mutagenic effects of RF-EMF at the DNA sequence level. In conclusion, the provided NGS analysis to characterize rat gliomagenesis is not yet technically convincing enough to be used in further investigations. From a radiation protection point of view, this study does not provide compelling evidence for an aetiological relationship between RF-EMF exposure and tumorigenesis in the RI study, as claimed by the authors.

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Impressum

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urn:nbn:de:0221-2024061144258

Spotlight - Jun/2024 no.2 (Eng)