Proposal for Modification of Cahan's Criteria Utilizing Molecular Genetic Analyses for Cases without Baseline Histopathology: A Unique Method Applicable to Primary Radiosurgery

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Abstract

Background Cahan's criteria have been utilized since 1948 to establish causality between prior radiation treatment and the development of secondary malignancy. One major criterion specifies that histological and radiographic evidence collected before and after radiation treatment must confirm separate tumor types; however, pretreatment biopsy is rarely obtained prior to radiosurgery for vestibular schwannoma and many other skull base and cranial lesions. Therefore, in these cases Cahan's criteria cannot be validly applied.

Objective This article proposes an update to Cahan's criteria using modern molecular genetic analysis for cases lacking baseline histopathology.

Methods Mate-pair sequencing and whole exome sequencing of a cerebellopontine angle undifferentiated high-grade pleomorphic sarcoma (UHGPS) that developed after stereotactic radiosurgery of a presumed benign vestibular schwannoma.

Results Mate-pair sequencing and whole exome sequencing of the sarcoma revealed complex chromosomal aberrations. Notably, the tumor contained a deletion in the *NF2* gene at 22q12 and an in-frame deletion on exon 5 of the remaining copy of *NF2*. Biallelic events impacting *NF2* are atypical for UHGPS but are characteristic for vestibular schwannoma. These findings help support the conclusion that the UHGPS arose from a benign vestibular schwannoma all along.

Conclusions Next-generation sequencing can be successfully applied to a radiation-induced sarcoma when both the original and malignant tumors harbor separate signature genetic markers. As our understanding of the genetic profile of various tumors expand, we believe that next-generation sequencing and other genomic tools will play an increasingly important role in establishing causality between radiation and the development of secondary malignancy.

Keywords

- Cahan's criteria
- radiation-induced malignancy
- radiosurgery
- Gamma-Knife
- sarcoma
- vestibular schwannoma

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Introduction

Radiation therapy has been an effective treatment of many benign and malignant lesions since inception in the late 19th century.^{1–4} However, it was not long before the risk of radiation-induced tumorigenesis was first recognized. Radiation-induced malignancies were initially described by Freiben et al in 1908⁵ and subsequently, in 1948, Cahan et al⁶ outlined the first diagnostic criteria for radiation-induced sarcoma (RIS). These criteria were rapidly adopted and modified to not only apply to sarcomas arising in irradiated bone, as was originally described, but to all malignancies that develop within the field of prior ionizing radiation. Murray et al⁷ modified and expanded upon these criteria to clarify the latency period between radiation and malignancy. These modified criteria are the version universally applied today and include (1) the tumor must develop within the margins of the previously irradiated tissue; (2) histological and radiographic evidence collected before and after radiation treatment must confirm separate tumor types; (3) a significant latency period must exist between irradiation and RIS, typically longer than 5 years; and (4) the patient cannot have a predisposing condition to tumorigenesis.

While these criteria have been effectively used to determine RIS for many years, current advancements in neurodiagnostic imaging and radiation delivery techniques used to treat many benign and malignant intracranial lesions have made criterion 2 difficult to achieve or adhere to. Specifically, many intracranial and skull base lesions can be accurately diagnosed and characterized on contrast enhanced magnetic resonance imaging (MRI) and/or thinly sliced computed tomography, and very rarely undergo histopathological confirmation via biopsy before treatment with Gamma-Knife (Elekta AB, Stockholm, Sweden), LINAC (e.g., Novalis [Brainlab, Munich, Germany] or CyberKnife [Accuray, Sunnyvale, California, United States]), or proton beam therapy. As these treatments are being more widely adopted, fewer patients are electing to undergo surgical resection as a baseline treatment. The true risk of radiation-induced malignancy after low-dose highly conformal radiation delivery remains unknown and highly contentious. Assessment of this risk is mired by the fact that most cases cannot completely fulfill Cahan's criteria without preradiation histological review. For example, in the case of a diagnosed malignancy after radiosurgery for a presumed benign vestibular schwannoma (VS), it remains unknown whether the original tumor was malignant all along, or if malignancy developed after treatment as a result of radiation effect or merely by pure coincidence.

Next-generation sequencing (NGS) has allowed us to efficiently and economically sequence whole exomes and genomes of human tissue with incredible accuracy. This technology has already been applied to genetic screening to evaluate for molecular predispositions to cancer and other diseases. Further, it has allowed us to examine the genetic profile of many benign and malignant neoplasms. For example, the National Cancer Institute and National Human Genome Research Institute have partnered to create The Cancer Genome Atlas, which has already generated compre-

hensive genetic profiles for 33 cancer types. With data generated from this initiative and other related work, we have the ability to rapidly and objectively determine the precise identity and lineage of a tumor. Based on this, we propose that NGS can be applied to investigate the association between radiation treatment and subsequent diagnosis of malignancy in cases where pretreatment histopathology is unavailable. Herein, we present a case example where this methodology is applied and discuss the utility of the strategy in the context of prior published cases.

Methods

IRB/Ethics Committee Approval and Patient Consent

Since 2006, the authors have collected and stored fresh frozen (–140°C) surgical waste specimens and matched plasma from consenting patients undergoing microsurgical resection of VS (International Review Board [IRB] #06–009116). Clinical details regarding the index case and a single control case were extracted from the electronic medical record (IRB#16–004498).

Mate-Pair DNA Library Preparation and Sequencing

Genomic deoxyribonucleic acid (DNA) from matched fresh frozen tumor and peripheral blood samples was isolated using the Qiagen Puregene extraction protocol (Qiagen, Hilden, Germany). The DNA was processed using the Illumina Nextera Mate Pair library protocol (Illumina, San Diego, California, United States) and sequenced on the Illumina HiSeq 2000 (Illumina, San Diego, California, United States). The sequencing produced 101-basepair paired-end fragments.

BMD Structural Variant Pipeline

The analysis pipeline for mate-pair sequencing (MPSeq) data are termed the BMD structural variant pipeline (Mayo Clinic, Rochester, Minnesota, United States). It was developed to find and report breakpoint junction locations and copy number variants (CNVs) from MPSeq data. The pipeline performs a two-step process on the sequencing data: alignment and structural variant analysis. Alignment is performed using the BIMA alignment method (Mayo Clinic, Rochester, Minnesota, United States).⁸ The mapped sequences are passed to the structural variant analysis tools module, which consists of a junction detection step and a CNV detection step (unpublished data, November 2017). The junction detection step returns breakpoint junctions resulting from large structural variants (e.g., deletions, tandem duplications, inversions, and translocations). The CNV detection step segments the genome into regions and statistically determines whether there is a loss or gain in genetic material in that region. These losses and gains either result from structural variants, which should be supported by corresponding breakpoint junctions, or result from whole-arm or whole-chromosome aneuploidy, where breakpoint junctions are not present or difficult to report.

Exome Sequencing and Variant Analysis

In this study, we utilized GenomeGPS 4.0 (Mayo Clinic, Rochester, Minnesota, United States), a workflow for genome

and exome data. This is a secondary analysis pipeline for NGS data. It includes three steps: alignment, single nucleotide and small insertion/deletion variant calling, and structural variation discovery and annotation. Unless otherwise specified, all tools were run under default configuration. FASTQ files were aligned to the GRCh38 reference genome Version 78 updated February 17, 2015⁹ using BWA 0.7.10 (Wellcome Trust Sanger Institute, Cambridge, United Kingdom)¹⁰ with the BWA-MEM algorithm for short read alignment. Realignment and recalibration were performed using GATK Version 3.4 (Broad Institute, Cambridge, Massachusetts, United States).¹¹ All germline variant calling (both single nucleotide and small insertions and deletions) were called through GATK Haplotype Caller and GenotypeGVCF walkers (Broad Institute, Cambridge, Massachusetts, United States). Somatic single nucleotide variations were genotyped using SomaticSniper (The Genome Institute, Washington University, St. Louis, Missouri, United States),¹² whereas insertions and deletions were called by GATK Somatic Indel Detector (Broad Institute, Cambridge, Massachusetts, United States).¹¹ Variant coding regions were functionally annotated using the BioR Version 2.5 annotation engine (Mayo Clinic, Rochester, Minnesota, United States).¹³

Results

Index Case of Presumed Radiation-Induced Sarcoma after Radiosurgery

To determine the viability of the proposed modification to Cahan's criteria, NGS was utilized to analyze the genetic profile of a previously reported case of an undifferentiated high-grade pleomorphic sarcoma (UHGPS or UPS; also known as malignant fibrous histiocytoma) that developed after stereotactic radiosurgery (SRS) of a presumed benign VS, which lacked preradiation histology.¹⁴ The patient was a 51-year-old man who presented with right-sided hearing loss. MRI studies revealed a tumor of the cerebellopontine angle and internal auditory canal with a maximum posterior fossa dimension of 2.7 cm (►Fig. 1A). Based upon characteristic imaging features, the presumptive diagnosis of VS was made. The patient was counseled regarding available treatment options and he elected to undergo primary Gamma-Knife radiosurgery. The treatment plan utilized a maximum dose of 30 Gy and marginal dose of 12 Gy and consisted of 22 isocenters to cover a volume of 19.1 cm³. Five months following treatment, he developed severe bifrontal headaches, ataxia, and blurry vision. Papilledema was noted on

Fig. 1 Axial T1-weighted gadolinium enhanced magnetic resonance images of tumor progression. (A) Diagnosed vestibular schwannoma at cerebellopontine angle measuring 4.3 cm before stereotactic radiosurgery (SRS). (B) Fluid-attenuated inversion recovery image obtained following SRS. (C) Six years following SRS showing tumor involution. (D) About 8.5 years following SRS showing tumor growth with internal hemorrhage. (Adapted from Schmitt WR, Carlson ML, Giannini C, Driscoll CL, Link MJ. Radiation-induced sarcoma in a large vestibular schwannoma following stereotactic radiosurgery: case report. *Neurosurgery*. 2011;68(3):E840–E846; discussion E846. Images republished with permission.)

fundusoscopic examination and MRI imaging confirmed obstructive hydrocephalus with the development of new peritumoral edema (►Fig. 1B). A ventriculoperitoneal shunt was subsequently placed, successfully alleviating his symptoms. At 6 years post-treatment, an MRI was performed as routine follow-up and revealed that the tumor had shrunk ~30% from 4.3 cm to 3.0 cm supporting a favorable treatment response (►Fig. 1C). However, 15 months later, the patient presented with progressing right facial nerve paralysis and trigeminal hypoesthesia in the V2 to V3 distribution. An MRI revealed that the tumor had grown 3.0 mm in the dorsocephalad dimension with apparent central hypodensity indicative of internal hemorrhage (►Fig. 1D). The patient underwent tumor resection and final pathology revealed the diagnosis of UHGPS based upon immunohistochemical analysis (►Fig. 2).

Application of Proposed Modification to Cahan's Criteria Utilizing Mate-Pair and Whole Exome Sequencing

NGS MPSeq and whole exome sequencing (NGS ExomeSeq) were applied to a sample of the UHGPS and were also applied to a typical VS specimen for illustrative comparison. Genomic plots were formed for both samples, which provide a visual account of structural changes of the chromosomes. The neurofibromatosis type 2 (*NF2*) gene on chromosome 22q is an established hallmark mutation in sporadic and syndrome-associated VS.^{15,16} The control case of a histologically confirmed benign VS revealed a loss of chromosome 22 containing the *NF2* gene at the 22q12 position, with the rest of the genome remaining largely intact (►Fig. 3). The

genomic plot from the UHGPS revealed complex chromosomal rearrangements and many copy number gains/losses. Notably, the tumor contained a deletion in chromosome 22 containing the *NF2* gene at 22q12 and an in-frame deletion on exon 5 of the remaining copy of *NF2*. Biallelic events impacting *NF2* are not typical for UHGPS but are characteristic for VS (►Fig. 4). Other areas of significant loss are chromosomes 3, 6, 8, 11, 12, 15, 16, 17, and 18. Chromosomes 2 and 4 showed significant genomic gain.

Mutations in the protein Merlin, encoded by the *NF2* tumor suppressor gene, have been strongly implicated in sporadic and *NF2*-associated VS tumorigenesis (►Table 1). Jacoby et al. and other authors have performed genetic analysis of numerous cohorts of VS using various methodologies and found that up to 86% of VS tumors contain an *NF2* mutation (►Table 1).

ExomeSeq analysis of a control case of a confirmed benign VS sample revealed a single nucleotide frame-shift deletion variant in the *NF2* gene (g.del29654678). This presumed pathogenic variant was one of only 24 variants that passed the high-confidence/high-impact filters. ExomeSeq analysis of the UHGPS in our previously reported index case revealed a nonrecurrent in-frame deletion variant in the *NF2* gene at position g.del29654668. This variant was one of only 35 that passed the high-confidence/high-impact filters. In both the confirmed VS control sample and the UHGPS sample, the MPSeq/ExomeSeq analysis demonstrates an *NF2* variant coupled with a loss of heterozygosity (LOH) in chromosome 22. Of note, the pathogenic deletions identified in both the VS and UHGPS were not identified in peripheral blood supporting that both were de novo somatic events. In both cases, this

Fig. 2 Histology demonstrating undifferentiated pleomorphic sarcoma. (A) Section demonstrates dense fibrosis with spindle or pleomorphic hyperchromatic nuclei with prominent nucleoli. (B) S-100 protein stain is only seen in entrapped fibers. (A, B) Black arrows show mitotic figures. (Adapted from Schmitt WR, Carlson ML, Giannini C, Driscoll CL, Link MJ. Radiation-induced sarcoma in a large vestibular schwannoma following stereotactic radiosurgery: case report. *Neurosurgery*. 2011;68(3):E840–846; discussion E846. Images republished with permission.)

Fig. 3 Mate-pair whole exome sequencing of a histopathology confirmed vestibular schwannoma. Red arrow indicates the loss of chromosome 22, which contains the NF2/Merlin gene.

Fig. 4 Mate-pair whole exome sequencing of an undifferentiated high-grade pleomorphic sarcoma reveals a loss of chromosome 22 (red arrow). Other areas of loss are on chromosomes 3, 6, 8, 11, 12, 15, 16, 17, and 18. Chromosomes 2 and 4 show significant genomic gain. Many translocations are present indicating a cellular dysmorphia.

Table 1 Mutational profiles of reported sporadic vestibular schwannomas

Series	Number of samples	Average age	Percentage with NF2 mutation	Percentage with LOH	Percentage with double hit	Additional identified genes
Jacoby et al, 1996 ¹⁷	50	48	86 (43/50)	47 (27/58) ^a	66 (38/58) ^a	–
Bian et al, 2005 ¹⁸	36	45.8	61 (22/36)	44 (16/36)	42 (15/36)	–
Stemmer-Rachamimov et al, 1997 ¹⁹	15	Not reported	60 (9/15)	47 (7/15)	50 (8/15)	–
Lee et al, 2012 ²⁰	30	48.3	60 (18/30)	30 (9/30)	30 (9/30)	–
Håvik et al, 2018 ²¹	46	51	76 (35/46)	22 (10/46)	35 (16/46)	CDC27, USP8
Chen et al, 2017 ²²	91	45	59 (54/91)	30 (27/91)	33 (30/91)	–
Agnihotri et al, 2016 ²³	13	45	85 (11/13)	62 (8/13)	62 (8/13)	SH3PXD2A-HTRA1 fusion
Lassaletta et al, 2013 ²⁴	51 ^a	48	49 (25/51)	57 (29/51)	45 (23/51)	–
Torres-Martin et al, 2013 ²⁵	29	44.5	72 (21/29)	59 (17/29)	Not reported	SPP1/MET
Hadfield et al, 2010 ²⁶	98	Not reported	66 (65/98)	56 (54/96)	Not reported	–
Welling et al, 2002 ⁴²	5	Not reported	66 (4%)	Not reported	Not reported	LUCA-15, SPARC
Aarhus et al, 2010 ⁴³	25	54	76 (19/25)	Not reported	Not reported	CAVI
Welling, 1998 ⁴⁴	29	54	66 (19/29)	Not reported	Not reported	Not reported

Abbreviations: LOH, loss of heterozygosity.

^aIncludes 8 NF2 cases that could not be delineated.

would result in the presence of a single, mutated allele of the *NF2* gene that was present in the tumor alone or in a limited number of somatic tissues. This is in line with current literature that demonstrates 22 to 62% of VS contain a LOH of chromosome 22.^{17–26} Furthermore, it has been reported that 30 to 66% of VS contain a double hit knockout of the *NF2* gene, either from mutations or a mix of mutation and LOH.^{17–24}

Conversely, previously reported cases of UHGPs have lacked such specific chromosomal aberrations or mutations in chromosome 22. In the investigation of the genetic profile of UHGPs, Lewin et al²⁷ and Perot et al²⁸ found that 22 and 71% of cases harbored a *TP53* mutation on chromosome 17, respectively. Chibon et al found that losses of the 13q14–q21 region were observed in 78% of tumors studied and implicated deletion of the *RB1* gene in tumorigenesis.²⁹ Additional recurrent losses or gains of genomic regions that have been reported in UHGPs include 1p36, 1q11, 1q21–q22, 3p12, 3p26.3, 7q22.1, 11p11, 17p11, 19p13, and 20q13.33.^{30–34}

Discussion

SRS is commonly employed for a wide range of benign and malignant intracranial and skull base lesions, such as VS, meningioma, pituitary tumors, metastases, and glioblastoma multiforme.^{35,36} While the occurrence of radiation-induced malignancy following conventional broad field external beam radiation has been well studied, the frequency at which this event occurs following SRS remains largely unknown and a topic of contentious debate. In this population, estimates are mired by the very low incidence of the event and the challenges of ascertaining causality using Cahan's criteria in the absence of pretreatment histology, as described by Loeffler et al.³⁷ Each item outlined in Cahan's criteria helps to establish increasing probability that the tumor in question developed as a result of prior irradiation. Therefore, the addition of independent determinants of causality will serve to further increase the diagnostic potential and utility of the criteria.

To address the limitation commonly encountered in cases of SRS—not having pretreatment histology available for review—we explored the application of NGS to analyze the genetic profile of the malignant tumor to determine whether it shared any common characteristic features with the alleged progenitor neoplasm. This strategy may be particularly advantageous in cases where the malignant tumor and the presumed originating tumor each have their own separate pathognomonic mutations (e.g., *NF2* gene in VS). Evaluating the case of malignancy developing after SRS for a sporadic VS as an example, we can assign different levels of probability of separate tumor types based upon the results of molecular genetic testing, with a caveat being that the patient does not harbor a *germline* pathogenic variant in the *NF2* gene detected in 100% of reads from a peripheral blood sample.

- *High probability that the malignant tumor arose from a benign VS:* when the newly diagnosed malignant tumor does not typically include a pathologic variant (mutation)

in the *NF2* gene based upon published data; however, the surgical specimen reveals a concomitant pathologic variant in the *NF2* gene.

- *Intermediate probability that the malignant tumor arose from a benign VS*: when the newly diagnosed malignant tumor contains a pathologic variant that has shown some evidence to be linked to VS, but it is either not exclusive to this tumor type or is not overwhelmingly seen.
- *Low probability that the malignant tumor arose from a benign VS (i.e., may have been a malignant tumor all along)*: when genetic analysis of the tumor specimen fails to identify any pathologic variant in the *NF2* gene or other genes implicated in VS such as *LZTR1*.
- *Indeterminate probability that the malignant tumor arose from a benign VS*: when the newly diagnosed malignant tumor is also known to commonly harbor a mutation on chromosome 22 and the *NF2* gene based upon published data.

In this study, we introduce genetic analysis as a new modification to Cahan's criteria by applying it to a previously reported case.¹⁴ In the original publication by Schmitt et al, the patient's presentation of unilateral hearing loss, no other associated cranial neuropathies, and MRI imaging demonstrating typical features of benign VS provided reasonable evidence that the tumor was in fact a benign schwannoma prior to radiation. However, as Kondziolka rightfully points out in an associated letter to the editor,³⁸ "because the original pathology was not known, this does not fulfill the Cahan criterion of a change in histology... Indeed, this tumor may have been an unrecognized sarcoma at presentation"—a fair criticism that plagues most other similar reports associated with SRS for VS.

With no available preirradiation histology to satisfy criterion two, we sought NGS to identify genomic aberrations supportive of molecular transformation from an antecedent benign schwannoma. As outlined in ►Table 1, *NF2*/merlin genetic mutations have been robustly linked to VS.³⁹ For example, *NF2* is caused by germline or somatic mosaic pathogenic variants in the *NF2* gene resulting in bilateral VS in almost all patients.⁴⁰ Further, our review of the literature found the most recent study indicating a 59% incidence of *NF2* genetic alterations in genomic DNA from sporadic VS with 33% demonstrating a double hit.²² We suspect that the rate of *NF2* mutations in VS is greater than 59%, and in our own experience we have found the number to approach 100% (*unpublished data*). Furthermore, our review of the literature found no published cases of UHGPs containing *NF2* gene mutations or other frequent chromosome 22 aberrations. Because MPSeq/ExomeSeq analysis of the index RIS demonstrated an *NF2* variant coupled with LOH in chromosome 22, based on a reasonable degree of scientific probability, we can conclude that the tumor was in fact originally a VS rather than being a sarcoma all along. Had the genetic analysis failed to identify any such variants, the evidence would strongly support that the malignancy did not arise from a VS.

This novel strategy of examining the molecular footprint of a newly diagnosed malignancy to help determine the

original tumor histology may be applied to other tumor types. Ewing sarcomas, for example, are almost 100% correlated with an EWS-FLI1 or EWS-ERG fusion transcript.⁴¹ This fusion transcript is found in no other tumor types and is highly correlative. Therefore, finding this fusion transcript in a RIS tumor sample would provide conclusive evidence of the original tumor identity.

Conclusion

The application of Cahan's criteria, and the determination of what can and cannot be fairly deemed a *radiation-induced* neoplasm, has been the subject of much debate. Cahan's criteria are comprised of somewhat imprecise criteria that have undergone several revisions corresponding to medical advances. Molecular genetic analysis may provide valuable information in cases where no preirradiation histopathology is available—an increasingly common scenario with the more widespread use of radiosurgery. In this report, we demonstrate that NGS and mate-pair analysis can be successfully applied to a RIS when both the original and malignant tumor harbor established separate signature genetic markers. As our understanding of the genetic profile of various tumors expand, we believe that NGS, mate-pair analysis, and other genomic tools will play an increasingly important role in establishing causality between radiation and the development of secondary malignancy.

Previous Presentations

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References

- 1 Pioneer in X-Ray Therapy. News of science. Science 1957;125 (3236):18–22
- 2 Williams F. *Roentgen Rays in Medicine and Surgery*. 2nd ed: New York, NY: Macmillan; 1902
- 3 Belot J. *Radiotherapy in Skin Disease*. London, United Kingdom: Rebman; 1905
- 4 Halperin EC, Perez CA, Brady LW, Wazer DE. *Perez & Brady's Principles and Practice of Radiation Oncology*. 6th ed: Philadelphia, PA: Lippincott Williams & Wilkins; 2013
- 5 Friebe H. Demonstration eines Cancroid des rechten Handrueckens, das sich nach langdauernder Einwirkung von Roentgenstrahlen entwickelt hatte. Fortschr Geb Rontgenstr 1902;6:106–111
- 6 Cahan WG, Woodard HQ, Higinbotham NL, Stewart FW, Coley BL. Sarcoma arising in irradiated bone; report of 11 cases. Cancer 1948;1(01):3–29
- 7 Murray EM, Werner D, Greeff EA, Taylor DA. Postradiation sarcomas: 20 cases and a literature review. Int J Radiat Oncol Biol Phys 1999;45(04):951–961
- 8 Drucker TM, Johnson SH, Murphy SJ, Cradic KW, Therneau TM, Vasmatzis G. BIMA V3: an aligner customized for mate pair library sequencing. Bioinformatics 2014;30(11):1627–1629

- 9 Harrow J, Frankish A, Gonzalez JM, et al. GENCODE: the reference human genome annotation for The ENCODE Project. *Genome Res* 2012;22(09):1760–1774
- 10 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25(14):1754–1760
- 11 McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 2010;20(09):1297–1303
- 12 Larson DE, Harris CC, Chen K, et al. SomaticSniper: identification of somatic point mutations in whole genome sequencing data. *Bioinformatics* 2012;28(03):311–317
- 13 Kocher JP, Quest DJ, Duffy P, et al. The Biological Reference Repository (BioR): a rapid and flexible system for genomics annotation. *Bioinformatics* 2014;30(13):1920–1922
- 14 Schmitt WR, Carlson ML, Giannini C, Driscoll CL, Link MJ. Radiation-induced sarcoma in a large vestibular schwannoma following stereotactic radiosurgery: case report. *Neurosurgery* 2011;68(03):E840–E846, discussion E846
- 15 Rouleau GA, Merel P, Lutchman M, et al. Alteration in a new gene encoding a putative membrane-organizing protein causes neurofibromatosis type 2. *Nature* 1993;363(6429):515–521
- 16 Trofatter JA, MacCollin MM, Rutter JL, et al. A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* 1993;72(05):791–800
- 17 Jacoby LB, MacCollin M, Barone R, Ramesh V, Gusella JF. Frequency and distribution of NF2 mutations in schwannomas. *Genes Chromosomes Cancer* 1996;17(01):45–55
- 18 Bian LG, Tirakotai W, Sun QF, Zhao WG, Shen JK, Luo QZ. Molecular genetics alterations and tumor behavior of sporadic vestibular schwannoma from the People's Republic of China. *J Neurooncol* 2005;73(03):253–260
- 19 Stemmer-Rachamimov AO, Xu L, Gonzalez-Agosti C, et al. Universal absence of merlin, but not other ERM family members, in schwannomas. *Am J Pathol* 1997;151(06):1649–1654
- 20 Lee JD, Kwon TJ, Kim UK, Lee WS. Genetic and epigenetic alterations of the NF2 gene in sporadic vestibular schwannomas. *PLoS One* 2012;7(01):e30418
- 21 Havik AL, Bruland O, Myrseth E, et al. Genetic landscape of sporadic vestibular schwannoma. *J Neurosurg* 2018;128(03):911–922
- 22 Chen H, Xue L, Wang H, Wang Z, Wu H. Differential NF2 gene status in sporadic vestibular schwannomas and its prognostic impact on tumour growth patterns. *Sci Rep* 2017;7(01):5470
- 23 Agnihotri S, Jalali S, Wilson MR, et al. The genomic landscape of schwannoma. *Nat Genet* 2016;48(11):1339–1348
- 24 Lassaletta L, Torres-Martín M, Peña-Granero C, et al. NF2 genetic alterations in sporadic vestibular schwannomas: clinical implications. *Otol Neurotol* 2013;34(07):1355–1361
- 25 Torres-Martín M, Lassaletta L, San-Roman-Montero J, et al. Microarray analysis of gene expression in vestibular schwannomas reveals SPP1/MET signaling pathway and androgen receptor deregulation. *Int J Oncol* 2013;42(03):848–862
- 26 Hadfield KD, Smith MJ, Urquhart JE, et al. Rates of loss of heterozygosity and mitotic recombination in NF2 schwannomas, sporadic vestibular schwannomas and schwannomatosis schwannomas. *Oncogene* 2010;29(47):6216–6221
- 27 Lewin J, Garg S, Lau BY, et al. Identifying actionable variants using next generation sequencing in patients with a historical diagnosis of undifferentiated pleomorphic sarcoma. *Int J Cancer* 2018;142(01):57–65
- 28 Pérot G, Chibon F, Montero A, et al. Constant p53 pathway inactivation in a large series of soft tissue sarcomas with complex genetics. *Am J Pathol* 2010;177(04):2080–2090
- 29 Chibon F, Mairal A, Fréneaux P, et al. The RB1 gene is the target of chromosome 13 deletions in malignant fibrous histiocytoma. *Cancer Res* 2000;60(22):6339–6345
- 30 Mandahl N. Cytogenetics and molecular genetics of bone and soft tissue tumors. *Adv Cancer Res* 1996;69:63–99
- 31 Choong PF, Mandahl N, Mertens F, et al. 19p+ marker chromosome correlates with relapse in malignant fibrous histiocytoma. *Genes Chromosomes Cancer* 1996;16(02):88–93
- 32 Rydholm A, Mandahl N, Heim S, Kreicbergs A, Willén H, Mitelman F. Malignant fibrous histiocytomas with a 19p+ marker chromosome have increased relapse rate. *Genes Chromosomes Cancer* 1990;2(04):296–299
- 33 Mertens F, Fletcher CD, Dal Cin P, et al. Cytogenetic analysis of 46 pleomorphic soft tissue sarcomas and correlation with morphologic and clinical features: a report of the CHAMP Study Group. *Chromosomes and Morphology. Genes Chromosomes Cancer* 1998;22(01):16–25
- 34 Silveira SM, Villacis RA, Marchi FA, et al. Genomic signatures predict poor outcome in undifferentiated pleomorphic sarcomas and leiomyosarcomas. *PLoS One* 2013;8(06):e67643
- 35 Mahboubi H, Sahyouni R, Moshtaghi O, et al. CyberKnife for treatment of vestibular schwannoma: a meta-analysis. *Otolaryngol Head Neck Surg* 2017;157(01):7–15
- 36 Persson O, Bartek J Jr, Shalom NB, Wangerid T, Jakola AS, Förander P. Stereotactic radiosurgery vs. fractionated radiotherapy for tumor control in vestibular schwannoma patients: a systematic review. *Acta Neurochir (Wien)* 2017;159(06):1013–1021
- 37 Loeffler JS, Niemierko A, Chapman PH. Second tumors after radiosurgery: tip of the iceberg or a bump in the road? *Neurosurgery* 2003;52(06):1436–1440, discussion 1440–1442
- 38 Kondziolka D. Radiation-associated sarcoma? *Neurosurgery* 2011;69(03):E786, author reply 787
- 39 Petrilli AM, Fernández-Valle C. Role of Merlin/NF2 inactivation in tumor biology. *Oncogene* 2016;35(05):537–548
- 40 Evans DG, Huson SM, Donnai D, et al. A clinical study of type 2 neurofibromatosis. *Q J Med* 1992;84(304):603–618
- 41 Delattre O, Zucman J, Plougastel B, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. *Nature* 1992;359(6391):162–165
- 42 Welling DB, Lasak JM, Akhmet'yeva E, Ghaheiri B, Chang LS. cDNA microarray analysis of vestibular schwannomas. *Otol Neurotol* 2002;23(05):736–748
- 43 Aarhus M, Bruland O, Sætran HA, Mork SJ, Lund-Johansen M, Knappskog PM. Global gene expression profiling and tissue microarray reveal novel candidate genes and down-regulation of the tumor suppressor gene CAV1 in sporadic vestibular schwannomas. *Neurosurgery* 2010;67(04):998–1019, discussion 1019
- 44 Welling DB. Clinical manifestations of mutations in the neurofibromatosis type 2 gene in vestibular schwannomas (acoustic neuromas). *Laryngoscope* 1998;108(02):178–189