Variants in DNA Repair Genes and Glioblastoma

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Background

- Glioblastomas – most common primary BT in adults
- Etiology largely unknown with no single risk factor identified that explains a substantial number of cases.
- Ionizing radiation – established environmental factor

US adult brain tumors

- 51,410 primary benign and malignant brain tumors diagnosed in 2007.

- ~ 20,500 primary malignant brain and CNS tumors.

CBTRUS

Incidence

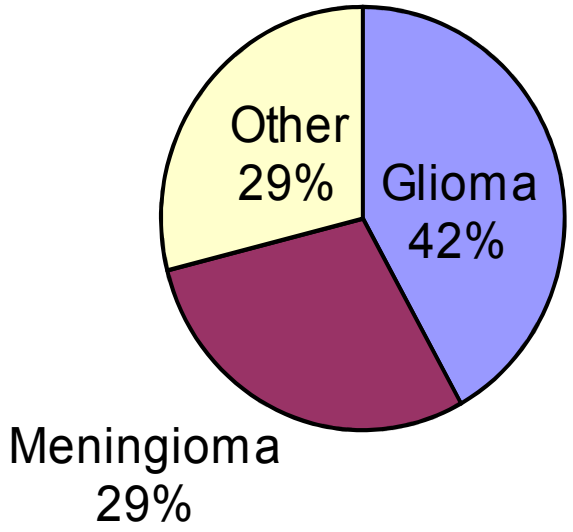
- Central Brain Tumor Registry of the United States (CBTRUS)

16.5 cases per 100,000 person per year *(2004-2007)*

non-malignant	9.2 cases per 100,000 person per year
malignant	7.3 cases per 100,000 person per year

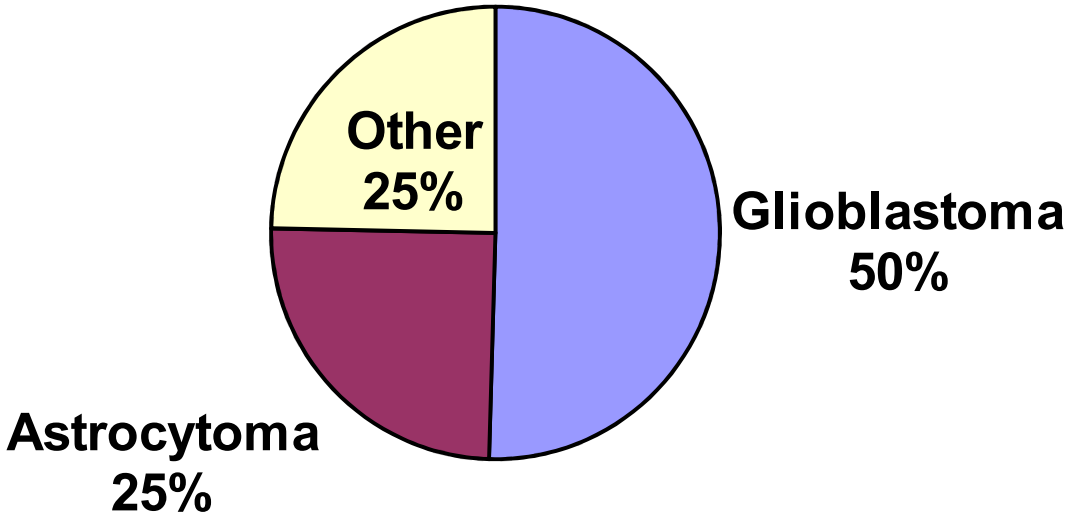
Age standardized to the 2000 US standard population.

Proportion of Brain Tumors by Histology

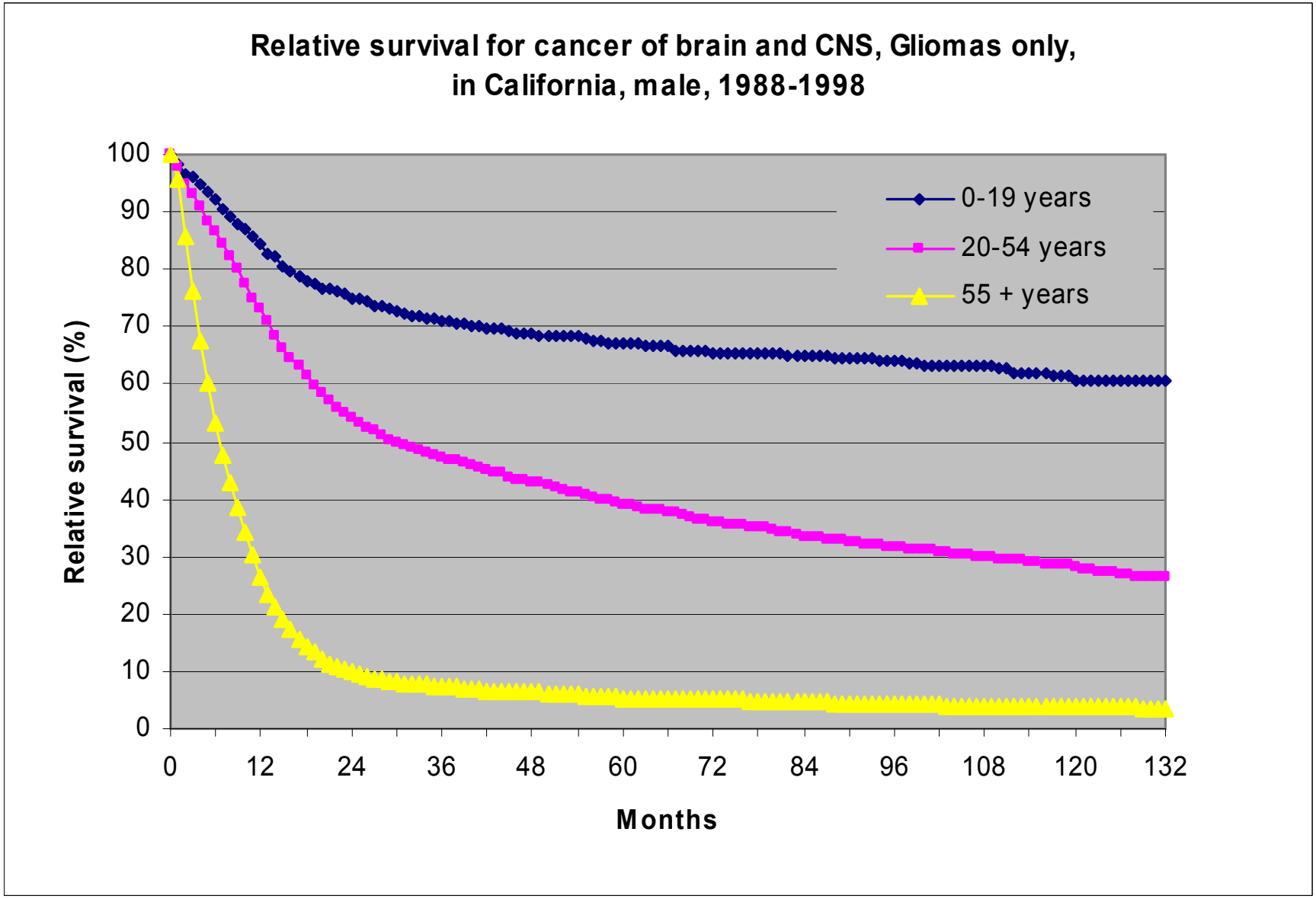


CBTRUS 04-05

Proportion of Gliomas by Histology



CBTRUS 2004-05


Relative survival for cancer of brain and CNS, Gliomas only,
in California, male, 1988-1998
Relative survival (%)
100
90
80
70
60
50
40
30
20
10
0
0
12
24
36
48
60
72
84
96
108
120
132
Months
0-19 years
20-54 years
55 + years

Challenges in Studying DNA Repair Genes and Glioblastoma

- Relatively rare disease
  - Sample size, power.
  - Need to combined samples from multiple centers
- Very poor survival
  - Representative sample
- Disease heterogeneity
  - Glioblastoma reasonably homogeneous group even without central neuropathology review

Brain Tumor Epidemiology Consortium (BTEC)

- open scientific forum organized to foster the development of multi-center, international and inter-disciplinary collaborations that will lead to a better understanding of the etiology, outcomes, and prevention of brain tumors.

- The Consortium formed in 2003

- Initial meeting sponsored by the National Cancer Institute's (NCI) Division of Cancer Epidemiology and Genetics (DCEG)

- Annual meetings.

- Four working groups focusing on adult gliomas, meningiomas, pediatric brain cancers, and family studies.

Pilot Study of DNA Repair Genes and Glioblastomas

Hypothesis

Genetic variation in DNA repair pathways could predispose adults to develop a GBM by influencing susceptibility to cellular damage that occurs as part of normal biological processes or susceptibility to environmental exposures.

Approach

- Collaborative study of Glioblastoma
- 4 collaborating centers identified through BTEC
- Basis for future collaborations on grants, genetic analyses.
- Funded by NBTF

Challenges of collaborative project

- **Data ascertainment.** Differences in recruitment, survival, case & control definitions.
- **Data Pooling.** Requirements of investigators, centers. Time & effort in data preparation
- **Standardization.** Comparability of studies. Different data collected, different formats.
- **Statistical Issue.** Small sample size for subgroup analysis.
- **Biospecimens.** Availability, handling, specimen quality.
- **Human Subjects, IRB, HIPPA.** Informed consent, sharing rules.

Methods

- Assemble samples from 4 existing case-control studies in the United States
  - MD Anderson, NCI, NIOSH, UCSF
- Create central dataset of DNA SNPs and relevant Qx data.
- Genotype GBM cases and controls with DNA available at each center.
  - Taq man assays at 3 centers.
- Incorporate Coriell control standards across centers.
- Complete combined analysis of association between each DNA repair variant and GBM.

Collaborating Centers

NIOSH:	Avima Ruder, PhD Mary Ann Butler, PhD
NCI:	Pete Inskip, PhD
MD Anderson:	Melissa Bondy, PhD
UCSF:	Margaret Wrensch, PhD John Wienke, PhD

Inclusion Criteria for Genotyping Study

Glioblastoma	ICD-O code 9440 *issues of central pathology review*
Ages	18 years and older
Race/ethnicity	NH White
Gender	Male and female
Location	One of 4 study centers (both population and hospital based)

Characteristics of 4 Studies

Characteristic	MD Anderson	NCI	NIOSH	UCSF
Study Design	CA center CA/CO	Hospital based CA/CO	Population-based CA/ CO	Population-based CA/CO
Control Selection Method	Hospital and population	Hospital	Population	RDD
Matching Factors	Age, gender, race.	Age, gender, race, hospital	Age, gender	Age, gender, race
Matching Type	Frequency	Frequency	Frequency	Frequency
Age Range	20-60 yrs	18 years+	18-80 years	20 years +
Years of diagnosis	1994-2000	1994- 1998	1995 –1997	1991- 1994, 1997- 1999

DNA repair candidates

Direct Repair,

Base Excision (BER),

Nucleotide Excision (NER),

Double Strand Break (DSB)

- Considered relevance of each repair pathway to the types of DNA damage that result from experimental neurocarinogens and from endogenous formation of reactive oxygen species (ROS).

  (Steve Hecht, MA Butler).

- Previous suggested associations from the literature, preliminary results from 4 collaborating centers, evidence of functional variant.

Each pathway responsible for efficient repair of specific types of DNA damage.

Base excision repair: multistep process for removal of small base adducts e.g. methylation or oxidation.

Nucleotide excision repair: corrects UV-induced lesions and bulky adducts

Direct repair: acts to reverse rather than excise DNA damage, typically involving methyl and other small alkyl groups.

Doublestrand breaks may occur following exposure to ionizing radiation or to products of cellular processes (hydrolysis, oxidation, or methylation of DNA).

Potential neurocarcinogens, associated DNA damage and relevant repair pathways.

Compound	Potential ROS formation?	Potential DNA damage	Relevant DNA repair pathways
Nitrosamides	No	alkyl adducts: (e.g. O^6-alkyl-thymine, O^4-methyl-guanine)	DR
Organophosphates	Yes	Does not appear to cause adducts or DNA breaks. Oxidative stress	BER, DSB-NHEJ, DSB-HRR
Organochlorines	Yes	Oxidative Stress	BER, DSB-NHEJ, DSB-HRR
Carbamates	No	alkyl adducts (form nitrosamides) O^6methylguanine Etheno (cyclic) adducts[41] (due to derivative); DNA double-strand break[(due to derivative)	DR, BER, DSB-NHEJ[a], DSB-HRR[a], NER[b]
Chlorinated Hydrocarbons	No	Etheno (cyclic) adducts (e.g. 7-(2-oxoethyl)-guanine (primary)	BER, DSB-NHEJ, DSB-HRR, NER[b]
Ionizing Radiation	Yes	Oxidative Stress; Double Strand Breaks	BER, DSB-NHEJ, DSB-HRR,

Candidate List

Table 1. Candidate DNA repair pathway genes, Glioblastoma Collaborative Group, 2008

Pathway	Gene name	Gene	SNP ID	SNP	Base change	Chr
Direct	Methyl-guanine methyltransferase	*MGMT*	rs12917	Leu84Phe	C/T	10q26.3
BER	8-Hydroxyguanine DNA glycosylase	*OGG1*	rs1052133	Ser326Cys	C/G	3p26.2
BER	Apurinic endonuclease	*APEX1*	rs1130409	Asp148Glu	T/G	14q11.2
BER	X-ray repair, complementing defective, 1	*XRCC1*	rs1799782	Arg194Trp	G/A	19q13.2
BER	X-ray repair, complementing defective, 1	*XRCC1*	rs25487	Arg399Gly	C/T	19q13.2
BER	ADP-ribosyltransferase	*PARP1*	rs1136410	Val762Ala	T/C	1q41
NER	Excision repair, complementing defective, 2	*ERCC2*	rs13181	Lys751Gln	A/C	19q13.3
NER	RAD23	*RAD23B*	rs1805329	Ala249Val	C/T	9q31.2
NER	Excision repair, complementing defective, 5	*ERCC5*	rs17655	His1104Asp	G/C	13q22
NER	Glioma tumor suppressor candidate region	*GLTSCR1*	rs1035938	Ser387Ser	C/T	19q13.3
NER	Excision repair, complementing defective, 1	*ERCC1*	rs3212986	C8092A	C/A	19q13.2
NHEJ	DNA-dependent protein kinase	*PRKDC*	rs7003908	6721G>T	G/T	8q11

Abbreviations: SNP, single-nucleotide polymorphism; ID, identification; Chr, chromosome; BER, base excision repair; NER, nucleotide excision repair; NHEJ, nonhomologous end–joining.

5 BER candidates: *OGG1, APEX1, XRCC1, PARP1*

5 NER candidates: *ERCC2, GLTSCR1, RAD23B, ERCC1*

1 NHEJ: *PRKDC (XRCC7)*

1 Direct: *MGMT*

Characteristics of CA CO in sample

Table 2. Characteristics of glioblastoma cases and controls, Glioblastoma Collaborative Group, 2008

	Case		Control	
	No.	(%)	No.	(%)
All sites	1,015		1,994	
MDA	213	(20.9)	365	(18.3)
NCI	171	(16.8)	489	(24.5)
NIOSH	139	(13.7)	453	(22.7)
UCSF	492	(48.5)	687	(34.5)
Gender				
Male	619	(61.0)	1,020	(51.1)
Female	396	(39.0)	974	(48.9)
Age*± SD	56.3 ± 12.6		53.6 ± 15.3	

*Age at diagnosis for cases and reference age for controls.

Statistical analyses

- Logistic regression of single SNP
  - DNA repair pathways
  - By age (<50 years, 50+ years)
  - By center

- Gene x Gene analyses by DNA repair pathway
  - focused interaction testing framework
  - all 12 SNPs; SNPs in specific pathways.

- Haplotype Chr 19q genes
  - ERCC2, ERCC1, GLTSCR1

Results

- All 12 SNPs in HW equilibrium

- 5 BER candidates: *PARP1*
- 5 NER candidates: no significant assns
- 1 NHEJ: *PRKDC*
- 1 Direct Repair: no sig associations

- No significant gene-gene interactions
- Haplotype effect for most common haplotype compared to all others.

Results

Gene SNP	genotype	CA[b] (%)	CO[b] (%)	OR[c]	95%CI
Base Excision Repair					
PARP1					
rs1136410					
	TT	713 (72.2)	1303 (67.3)	ref	
	CT	251 (25.4)	575 (29.7)	0.79	(0.67, 0.95)
	CC	23 (2.3)	57 (3.0)	0.83	(0.51, 1.38)
				p-trend=0.02	
Non Homologous End Joining					
PRKDC					
rs7003908					
	TT	389 (41.8)	811 (42.3)	ref	
	GT	397 (42.6)	875 (45.7)	1.07	(0.90, 1.27)
	GG	145 (15.6)	230 (12.0)	1.44	(1.13, 1.84)
				p-trend=0.009	

[c]adjusted for age, gender and study center.

Table 4. Haplotypes and risk for glioblastoma for loci at ERCC2, ERCC1, GLTSCR1, Glioblastoma Collaborative Group, 2008

	Haplotype*	Frequency	OR †, ‡	95% CI	*P*
H1b	ACC	0.39	0.77	0.61-0.98	0.04
H2	ACT	0.152	1.19	0.85-1.68	0.32
H3	AAC	0.065	0.7	0.49-1.22	0.2
H4	AAT	0.027	1.04	0.44-2.70	0.93
H5	CCC	0.161	1.19	0.85-1.67	0.31
H6	CCT	0.052	1.28	0.69-2.37	0.44
H7	CAC	0.109	1.17	0.77-1.77	0.46
H8	CAT	0.044	1.34	0.66-2.71	0.42

*Haplotypes for loci at ERCC2 rs13181, ERCC1 rs3212986, and GLTSCR1 rs1035938.

†Odds ratio for haplotype compared with all other haplotypes.

‡Adjusted for age, gender, and center.

9q13 SNPs *ERCC1, ERCC2, GLTSCR1*

Environmental and Lifestyle data

- Examine the potential use of questionnaire data from the four study centers for G x E.

- Explore occupational and environmental exposures and risk of GBM

- Variables:

History of Head Injury
Smoking History
Radiation Therapy

Occupational History
Family History of Cancer
Demographics

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Variants in the *CDKN2B* and *RTEL1* regions are associated with high-grade glioma susceptibility

Margaret Wrensch[1,2,12], Robert B Jenkins[3,12], Jeffrey S Chang[4,12], Ru-Fang Yeh[4,12], Yuanyuan Xiao[4], Paul A Decker[5], Karla V Ballman[5], Mitchel Berger[1], Jan C Buckner[6], Susan Chang[1], Caterina Giannini[3], Chandralekha Halder[3], Thomas M Kollmeyer[3], Matthew L Kosel[5], Daniel H LaChance[7], Lucie McCoy[1], Brian P O'Neill[7], Joe Patoka[1], Alexander R Pico[8], Michael Prados[1], Charles Quesenberry[9], Terri Rice[1], Amanda L Rynearson[3], Ivan Smirnov[1], Tarik Tihan[10], Joe Wiemels[2,4], Ping Yang[11,13] & John K Wiencke[1,2,13]

Discovery: Genome Wide Association study of high-grade glioma
- 692 glioma
- 3,992 controls (602 AGS and 3,390 Illumina icontrols)

Replication:
- 176 independent high grade glioma
- 174 controls

3 SNPs from discovery replicated
- 1 SNP near CDKN2B ($p= 3.4 \times 10^{-8}$)
- 2 SNPs in RTEL1 ($p= 3.4 \times 10^{-8}$)

Discovery only
- TERT

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Genome-wide association study identifies five susceptibility loci for glioma

Sanjay Shete[1,17], Fay J Hosking[2,17], Lindsay B Robertson[2], Sara E Dobbins[2], Marc Sanson[3], Beatrice Malmer[4], Matthias Simon[5], Yannick Marie[3], Blandine Boisselier[3], Jean-Yves Delattre[3], Khe Hoang-Xuan[3], Soufiane El Hallani[3], Ahmed Idbaih[3], Diana Zelenika[6], Ulrika Andersson[4], Roger Henriksson[4], A Tommy Bergenheim[7], Maria Feychting[8], Stefan Lönn[9], Anders Ahlbom[8], Johannes Schramm[5], Michael Linnebank[10], Kari Hemminki[11], Rajiv Kumar[11], Sarah J Hepworth[12], Amy Price[2], Georgina Armstrong[1], Yanhong Liu[1], Xiangjun Gu[1], Robert Yu[1], Ching Lau[13], Minouk Schoemaker[14], Kenneth Muir[15], Anthony Swerdlow[14], Mark Lathrop[6,16], Melissa Bondy[1] & Richard S Houlston[2]

- Meta-analysis of 2 GWAS studies Illumina 550K SNPs
- 1,878 cases
- 3,670 controls

- Replication:
- 2,545 cases
- 2,953 controls

- 5 SNPs risk loci for glioma

  TERT ($p= 1.5 \times 10^{-17}$) CCDC26 ($p=2.3 \times 10^{-18}$)

  RTEL1 ($p= 2.5 \times 10^{-12}$) CDKN2A-2B ($p= 7.2 \times 10^{-15}$)

  PHLDB1 ($p= 1.1 \times 10^{-8}$)

Discovery Genes

- *TERT*
  - Encodes human telomerase
  - A polymerase that maintains telomere ends
  - Activity elevated in glioblastoma
  - Influences glioma cell growth

- *RTEL1*
  - Encodes DNA helicase
  - Critical for regulation of telomere length
  - Loss associated with shortened telomere length, chromosomal breaks, & translocations

DNA Pilot Genes

- *PARP1*
  - Potential telomere-length regulator.
  - Role in detection of DNA damage.
  - Contributes to programmed cell death and up regulation of inflammatory responses.
  - PAR inhibitors non-toxic to normal cells cytotoxic to HR-defective cancer cells.
  - Current clinical trials for gliomas.

Conclusions

- Collaborative effort to combine data for rare cancer.
- Findings suggest that DNA repair variants may play important role in etiology of GBM.
- Outside studies suggest DNA repair pathway may be important pathway to improve treatment sensitivity.
- Large collaborations essential for genetic studies of rare cancers.
- Careful planning need to assure that comparable data collected for genetic and lifestyle factors.

MGMT O-6-methylguanine–DNA methyltransferase

- Epigenetic silencing of the *MGMT* DNA repair gene by promoter methylation compromises DNA repair and has been associated with longer survival in patients with glioblastoma who receive alkylating agents.

- The *MGMT* gene is located on chromosome 10q26 and encodes a DNA-repair protein that removes alkyl groups from the O-6 position of guanine, an important site of DNA alkylation.

- Temozolomide is a DNA methylating agent that induces a variety of methyl adducts, and failure to repair key methylation lesions results in significantly enhanced tumor cell death.

- Patients with glioblastoma containing a methylated *MGMT* promoter benefited from temozolomide, whereas those who did not have a methylated *MGMT* promoter did not have such a benefit.

Heigi, N Engl J Med 2005;352:997-1003.