Signaling Pathways in the Relapse of Glioblastoma

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ABSTRACT

Background: Glioblastoma is the most common primary neoplasm of the central nervous system. Standard treatment includes surgery with maximum safe resection and radiotherapy plus concomitant and adjuvant chemotherapy; however, almost invariably, tumor relapse occurs. We aimed to describe signaling pathways and molecular mechanisms present in tumor relapse of glioblastoma.

Methods: This systematic review followed the PRISMA guidelines. We searched the PubMed, EMBASE and Web of Science databases. We included studies that enrolled patients 15 years or older with a diagnosis of glioblastoma according to Louis criteria and focused on signaling pathways and molecular mechanisms present in tumor relapse of glioblastoma. The outcome of interest was progression-free survival.

Results: We identified 1470 articles; 31 met the inclusion criteria. From each publication, we obtained the associated markers O-6-methylguanine-DNA methyltransferase, isocitrate dehydrogenase, mRNA, epidermal growth factor receptor (EGFR), p53, and others. All publications were evaluated with the Q-Genie checklist tool for quality assessment.

Conclusions: We identified a wide variety of signaling pathways and molecular processes that are involved in glioblastoma relapse. This diversity would explain intra- and intertumor heterogeneity, treatment evasion, and relapse. However, only a few molecular processes have robust evidence for clinical utility.

INTRODUCTION

Glioblastoma (GB) is the most common primary neoplasm of the central nervous system, with an overall incidence of 0.59 to 3.69 per 100,000.[1] It has a very poor prognosis, with a mean survival of 12 to 15 months from its diagnosis; only 3% to 5% of patients survive after 3 years. Standard treatment, based on the Stupp protocol,[2] includes surgery with maximum safe resection and radiotherapy plus concomitant and adjuvant chemotherapy. Despite this, almost invariably, tumor relapse occurs. In GB, unlike in other types of cancer, mortality is not associated with the appearance of metastasis but rather with tumor relapse, given the tumor's ability to evade treatment and the limitation in many cases for optimal resection of the neoplasm by the eloquence of the affected areas. Understanding the mechanisms used by the tumor to evade treatment is fundamental to finding new therapeutic targets. Our objective was to describe the signaling pathways and molecular mechanisms present in tumor relapse of GB considering the evolutionary processes of cancer described by Hanahan and Weinberg.[3]

METHODS

The search followed the declaration of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020.[4]

Inclusion and exclusion criteria

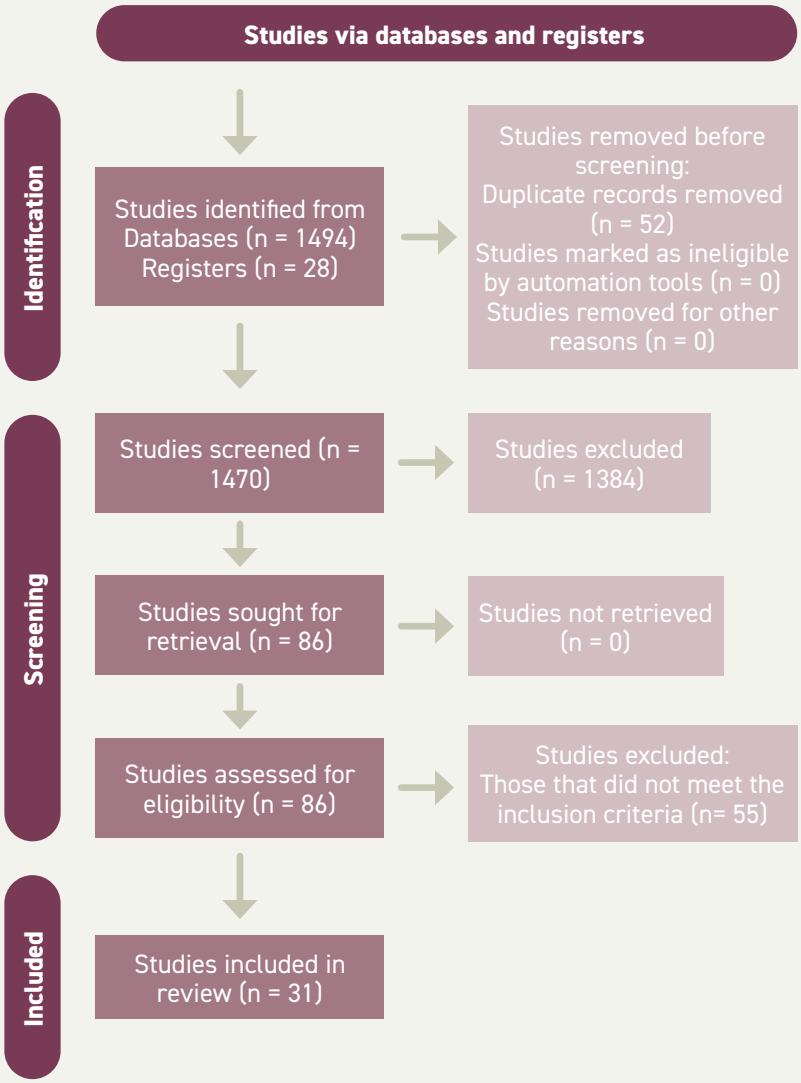
We included observational, case-control, and retrospective or prospective cohort studies that enrolled patients aged 15 years or older with a GB diagnosis according to the Louis criteria of 2007 updated in 2016; the patients must have received frontline treatment with surgery, followed by chemotherapy (Stupp protocol or otherwise, as indicated by the author) and/or radiotherapy, and must subsequently have experienced tumor relapse. The type of intervention analyzed in each article corresponded to which signaling pathways and molecular mechanisms could have been involved in disease progression according to the cancer hallmarks identified by Hanahan and Weinberg.[3] Exclusion criteria included those studies that, at the time of evaluation, did not have information on progression-free survival (PFS). The expected outcome was

TABLE 1. **Q-Genie Quality Assessment Scores for the Included Studies**

Studies	Items											Total
	1	2	3	4	5	6	7	8	9	10	11	
Cheng et al (2015)	4	5	3	2	5	3	3	7	3	4	4	43
Yin et al (2018)	4	5	3	4	3	3	3	7	4	4	6	46
Marziali et al (2017)	6	4	5	5	3	3	2	3	5	4	2	42
Adam et al (2012)	2	4	3	5	3	3	2	5	3	4	7	41
Weller et al (2014)	3	5	3	4	3	3	3	5	2	4	6	41
Dréan et al (2018)	6	4	5	5	4	3	3	3	6	4	4	47
Ohba et al (2019)	6	3	5	4	5	3	2	5	5	4	6	48
Swellam et al (2019)	6	3	4	5	4	3	3	4	5	4	5	46
Lalezari et al (2013)	6	5	3	4	3	3	3	3	3	4	6	43
De Carlo et al (2018)	6	5	3	5	3	3	2	3	5	4	6	45
Tini et al (2018)	3	5	3	4	3	3	3	6	4	4	3	41
Meng et al (2014)	4	6	5	3	5	3	3	5	5	4	7	50
Toraih et al (2019)	6	5	4	5	4	3	2	5	2	4	7	47
Zhang et al (2016)	3	4	4	5	4	3	3	4	4	4	6	44
Li et al (2008)	6	3	4	5	4	3	3	3	4	4	3	42
Kim et al (2017)	6	5	4	3	4	3	3	5	4	4	5	46
Dong et al (2017)	3	4	4	5	4	3	2	4	3	4	3	39
Wang et al (2017)	5	5	4	4	4	3	3	5	4	4	5	46
Pinel et al (2017)	6	4	4	4	3	3	3	5	3	4	5	44
Eoli et al (2017)	6	5	3	4	3	3	2	4	3	4	4	41
Kim et al (2012)	3	5	4	3	4	3	3	4	4	4	4	41
Kim et al (2017)	6	5	4	4	4	3	3	5	4	4	5	47
Shastry et al (2016)	5	6	3	3	4	3	2	5	5	4	6	46
Fan et al (2013)	5	4	3	3	4	3	3	5	4	4	6	44
Griguer et al (2013)	5	6	3	4	4	3	2	4	3	4	6	44
Limam et al (2019)	3	6	4	5	3	3	3	4	3	4	5	43
Lee et al (2013)	3	6	3	3	4	3	2	4	5	4	4	41
Wang et al (2014)	6	6	3	4	3	3	3	5	5	4	3	45
Tabouret et al (2015)	6	4	3	6	3	3	2	4	3	4	6	44
Sana et al (2014)	3	5	4	4	4	3	3	4	3	4	6	43
Olmez et al (2014)	6	3	3	3	3	3	3	3	3	3	3	36

Items: (1) justification of the study, (2) selection and definition of the outcome of interest, (3) selection and comparability of comparison groups, (4) technical classification of the exposition, (5) no technical classification of the exposition, (6) other sources of bias, (7) sample size and power, (8) classification of a priori analysis, (9) statistical methods and confusion control, (10) test of assumptions and interferences for genetic analysis, (11) suitability of the inferences drawn from the results.
Score: 1 to 7 (1 poor, 7 excellent).

FIGURE. **2020 PRISMA Flow Diagram**



relapse, defined as the reappearance of tumor lesions documented by images and confirmed by histology. PFS was sought as a criterion for this outcome.

Search strategy

On March 3, 2020, a systematic search was performed in the PubMed, EMBASE, and Web of Science databases, from January 2004 through December 2019; the late end date ensured the inclusion of the most relevant literature at the time. Search terms were selected based on the population/exposure/outcome (PEO) framework and combined using Boolean operators ("AND", "OR"). Filters were used to limit the results to those using human subjects, written in English, and published within the desired time frame. These terms were used in the search strategy: glioblastoma, methyltransferase, tumor suppressor gene, phosphatidylinositol, cell senescence proto-oncogene, neurofibromin, sustaining proliferative signaling, avoiding growth suppression, avoiding immune destruction, tumors promoting inflammation, dysregulation of cellular energy, DNA repair enzymes, regulatory genes of gene expression, tumor suppressor, signal transduction, genomic instability, cell reprogramming, phosphatidylinositol 3-kinases, *ERB-1* genes.

Study selection and data extraction

After removing duplicates, titles and abstracts were screened for their relevance to the scope of this review. Two authors, J.O. and F.P., independently assessed the eligibility of retrieved articles. To determine suitability for inclusion in this review, the full text of each potentially relevant article was analyzed for overall content and compliance with the eligibility criteria. The following data were extracted from each eligible article: authors, year of publication, study design, population studied, molecular process, diagnostic method, and main findings. The outcome of interest was PFS or time to progression (TTP). Disagreements were resolved by consensus.

Quality and risk of bias assessment

The quality and risk of bias of the studies were assessed through the Q-Genie tool, developed by the Quality of Genetic Association Studies.[5] Q-Genie consists of 11 items. Each item was rated using a 7-point Likert scale (1 = poor; 2-3 = good; 4-5 = very good; 6-7 = excellent). The overall quality of the articles was classified by comparing the scores for each topic. Studies were classified as good, moderate, or poor quality if their scores were greater than 40, between 32 and 40, and less than 32, respectively.

Synthesis methods

HRs were extracted, along with their 95% CIs and *P* values. For continuous

TABLE 2. **Characteristics of the Included Studies**

Reference	Country	N	Age range (yrs)	Mean age (yrs)	Factor	
Cheng et al (2015)	China	19	ND	ND	miR-222, -145, -20a, -132, -129	
Yin et al (2018)	France (RAUH) Neurosurgery Departments of Rennes and Angers University Hospitals	79	36-75	58.9	Epigenetic silencing (TRIM58, ADRA2C); restatement (TRIM38, MS4A7);	
Yin et al (2018)	Canada/Germany (GSE36278-Gen expression omnibus series)	57	18-57	42.2	Epigenetic silencing (TRIM58, ADRA2C); restatement (TRIM38, MS4A7);	
Marziali et al (2017)	Italy	35	30-80	59.5	miR-23a, miR-27a, miR-9 expression	
					MGMT methylation	
					EGFR vIII overexpression	
					PTEN little expression	
					VEGF overexpression	
Adam et al (2012)	Germany	60	33-86	61	ALDH1A1 expression	
					MGMT methylation	
Weller et al (2014)	Germany	179	24-84	60.6	EGFR vIII overexpression	
Dréan et al (2018)	France	51	ND	ND	ABCA13 overexpression	
					MGMT methylation	
Ohba et al (2019)	Japan	59	17-86	54.5	cMET overexpression	
Swellam et al (2019)	Egypt	20	>18	60	miR-221 overexpression	
					miR-222 overexpression	
Lalezari et al (2013)	USA	355	22.3- 90.0	57.6	MGMT ≥30 vs <30%	
					IDH1 not mutated	
					MGMT methylation	
					IDH1 not mutated	
					MGMT BISEQ ≥3 vs <3	
					IDH1 not mutated	

Abbreviations: ADN, methyltransferases; CcO, cytochrome oxidase; cMET, hepatocyte growth factor receptor; CXCL12, CXC motif chemokine ligand 12; CXCR, chemokine receptor family; DGKI, diacylglycerol kinase iota; DNA-PKcs, DNA-dependent protein kinase; DNMT, DNA methyltransferases; GLI1, glioma-associated oncogene homologue 1; HIF1a, inducible factor for hypoxia; HMT, histone methyl transferase; IDH, isocitrate dehydrogenase; IFIT1, interferon-induced protein with tetratricopeptide repeats 1; LAPTM4B-35, lysosomal protein transmembrane 4 beta; lncRNA, long noncoding RNA; MDM2, murine double minute 2; MGMT, O-6-methylguanine DNA methyltransferase; miR, microRNA; MS_P, statistical significance log rank test;

	Method	MSpos (mo)	MSneg (mo)	MSdif (mo)	MSDIF CI	MS_P	HR	HR, 95% CI
	Chinese Glioma Genome Atlas; high vs low risk mirVana miRNA Isolation 27 Kit	4.3	22.1	17.8	ND	0.0113	3.39	1.44-12.91
	Infinium Human Methylation 450k platform (Illumina Inc)	10.3	13.9	3.6	ND	0.008	ND	ND
	Infinium Human Methylation 450k platform (Illumina Inc)	8.3	48	39.7	ND	0.0001	ND	ND
	qPCR	5	4	1	ND	0.4921	ND	ND
	MSP	14	8	6	ND	0.0167	ND	ND
	IHQ	8	10.5	2.5	ND	0.5389	ND	ND
	IHQ	9	8	1	ND	0.0284	ND	ND
	IHQ	9	3	6	ND	0.1459	ND	ND
	IHQ	8	6	2	ND	0.267	0.826	0.48-1.41
	MSP	11	6	5	ND	0.016	0.516	0.29-0.92
	MLPA, IHQ, RTPCR	7.4	6.6	0.8	ND	0.803	ND	ND
	Accutase StemPro RT qPCR, IHQ	ND	ND	ND	ND	0.0064	1.12	ND
	MSP	ND	ND	ND	ND	ND	3.13	ND
	IHQ	5.3	8.3	3	ND	0.045	ND	ND
	miRNeasy Mini kit qPCR	7.3	10.4	3.1	ND	0.001	ND	ND
	miRNeasy Mini kit qPCR	7.6	10.4	2.8	ND	0.0001	ND	ND
	IHQ	7.8	10.9	3.1	ND	0.0001	1.49	1.18-1.89
	ND	ND	ND	ND	ND	0.0436	0.54	
	MSP	13.3	7.8	5.5	ND	0.0001	0.53	0.42-0.67
	ND	ND	ND	ND	ND	0.081	0.62	0.36-1.06
	BiSEQ	11.5	7.9	3.6	ND	0.0001	0.64	0.49-0.82
	ND	ND	ND	ND	ND	0.1162	0.54	0.29-0.99

Abbreviations Continued: MS4A7, membrane spanning 4-domains A7; MSdif, median survival difference; MSneg, mean survival in negative (unexposed); MSpos, mean survival in positive (exposed); N, number of patients; ND, no data; P53, protein 53; PTCH, patched; PP1A, nuclear protein phosphatase 1; PTEN, phosphatase and tensin homologue; SLC7A7, solute carrier family 7 member 7; TCTN1, tectonic family member 1; TRIM38, tripartite motif containing 38.

To view the full Table 2, visit **cancernetwork.com/Glioblastoma_3.23**

results, we extracted data from means, standard deviations, and the number of participants in each group. For continuous asymmetric data, data from medians, ranges, and *P* values were extracted from nonparametric tests. All results are presented in tables.

RESULTS

Study selection

We identified 1470 articles. After the addition of filters, duplication removal, eligibility screening, and final selection, 31 studies were included (**Figure**).

Quality assessment

A detailed quality rating for each of the 31 articles is shown in **Table 1.** Two were classified as moderate quality and 29 were classified as good quality; all scored between 36 and 50 on the Q-Genie checklist. In the itemized analysis, we found that most publications have "very good" and "excellent" scores in the following: selection of the working hypothesis, selection and definition of the outcome of interest, a priori planning of the analysis, and ideal inference extracted from the results. The items "statistical methods" and "selection of groups" were generally scored as "good" on the Likert scale (Table 1).

Study and subject characteristics

The study types included were cross-sectional, cohort, and observational follow-up. **Table 2** shows all characteristics of included studies. **Table 3** presents a detailed description of the signaling pathways and molecular processes that are involved in the relapse of GB. A total of 3585 subjects participated in the 31 studies, with a mean age of 56.05 years (range, 15-90) at the time of GB relapse.

Molecular pathology

The publications describe the processes of deparaffinization, DNA recovery and RNA recovery with different kits, immunohistochemical (IHC) techniques, quantitative polymerase chain reaction (qPCR) with TaqMan and SYBR green probes, multiplex-ligation dependent probe amplification, and high-resolution melting, among other laboratory techniques. The methylation status of O-6-methylguanine DNA methyltransferase (MGMT) was performed in most cases by methylation-specific PCR (MSP) and in others by pyrosequencing (PyroMark Q96 ID). CpG island methylation was measured with Infinium Human Methylation 450k platform (Illumina Inc) and VeraCode GoldenGate Methylation technology (Illumina Inc), among others. The expression of micro-RNA was obtained by mirVana miRNA Isolation 27 Kit and miRNeasy Mini kit; pyrosequencing is the most widely used sequencing technique.

Outcomes

The PFS or TTP was defined by each article as the time between the first surgery and the appearance of a new lesion in imaging studies using McDonald criteria.[5] In some previous studies, PFS was measured but not defined, and the outcomes were described as median survival times, which were compared using the log rank test. Some included studies estimated HRs through Cox regression. Quantitative analysis is shown in Table 2.

O-6-methylguanine DNA methyltransferase (MGMT)

MGMT was reviewed in 21 publications. When the method used to identify MGMT methylation status was immunohistochemistry (IHC), Lalezari et al.[6], Zhang et al,[7] and Wang et al[8] reported statistically significant differences ($P \leq .05$) in the overall survival of patients with MGMT methylation present, with HRs between 1.49 and 2.162. Limam et al[9] did not find statistical significance through this method.

Marziali et al,[10] Adam et al,[11] Dréan et al,[12] Lalezari et al,[6] Tini et al,[13] Kim et al,[14] Eoli et al,[15] Kim et al,[16] Wang et al,[17] Lee et al,[18] Limam et al,[9] Kim et al,[19] and Sana et al.[20] measured MGMT status by MSP and reported statistically significant differences ($P \leq .05$). When the exposure factor was MGMT methylation, they obtained HRs between 0.45 and 0.59; when the exposure factor was unmethylated MGMT, they reported HRs between 1.6 and 2.505.

Limam,[9] who did not obtain statistical significance when using IHC, acquired statistically significant differences (HR, 0.096; 95% CI, 0.02-0.46; $P = .0001$) with MSP. Toraih et al,[21] Sadones et al,[22] and Pinel et al.[23] did not find significant differences.

Isocitrate dehydrogenase

Lalezari et al,[6] De Carlo et al,[24] Etcheverry et al,[25] Wang et al,[17] studied the effect of isocitrate dehydrogenase (*IDH*) with pyrosequencing, finding statistically significant differences for the mutated state, with HRs of 0.12, 0.42, and 0.62, and for the nonmutated state, with an HR of 4.1. Kim[19] found no differences using IHC.

Micro-RNA

Cheng et al,[26] Swellam et al,[27] and Sana et al,[20] investigated micro-RNA (miR) signatures, finding statistically significant differences. Cheng et al[26] used the mirVana miRNA Isolation 27 Kit, finding overexpression of miR-222, -132, and -129 in the CGGA (Chinese Glioma Genome Atlas), with an HR of 3.39. Swellam et al[27] evaluated miR-221 and -222 by qPCR. Sana[20] observed that miR-224, miR-432, miR-454, and miR-672 were overexpressed, while miR-31 and miR-885-5p were under expressed (TaqMan Array Human MicroRNA A + B Cards Set v3.0), with an HR of 1.98.

Epidermal growth factor receptor

Tini et al[13] measured epidermal growth factor receptor (EGFR) expression by

TABLE 3. **Summary of Molecular Processes With Statistical Significance That Interfere in Each of the Capacities Acquired and Necessary for Tumor Growth and Progression, Stratified According to Cancer Hallmarks**

Molecular process	Acquired capacities										Strategy
	1	2	3	4	5	6	7	8	9	10	
MGMT			X				X				The silencing of this repair mechanism allows the accumulation of chemotherapy-induced damage, which generates cell death as long as the cell death mechanisms are intact, or instability if they are not.
miR-222	X					X	X				Overexpression, associated with silencing of MGMT, but also with greater invasion
miR-145						X					Lower expression associated with adducin 3 (ADD3) in the cytoskeleton and SOX9 involved in initiation and progression in solid tumors, and resistance to prostate cancer therapy
miR-132					X				X		Overexpression, promoter of angiogenesis and inflammation
miR-129	X					X					Lower expression, considered tumor suppressor
miR-20a	X										Overexpression in glioblastoma stem cells
miR-23a	X					X					Overexpression, modulate PTEN MAX-interacting protein 1, suppressor of cMyc
miR-27a	X		X								Overexpression, regulates progression in the cell cycle
miR-9						X					Decreased expression, participates in mesenchymal differentiation by JAK/STAT suppression
miR- 21 miR- 22	X					X					Overexpression, silencing PTEN, PUMA (pro-apoptotic), TIMP3 (metalloprotein inhibitor), activation of AKT
miR-485-3p	X		X			X					Decreased expression, tumor suppressor, associated with resistance to therapy due to its relationship with NFYB (beta subunit of nuclear transcription factor Y), NTRK3 (tropomyosin tyrosine kinase family)
miR-31	X					X					Low expression
miR-224	X					X					Overexpression, involved in the activity of CXCR4, metalloproteinase 1
miR-885-5p						X					Low expression
IFIT1	X		X				X				It is a gene induced by interferon α/β, which participates in the inhibition of MGMT, binds to the ribosomal protein L5 to inhibit tumor growth, and forms complexes with IFIT2/3 to induce apoptosis. IFIT overexpression would confer increased response to treatment.
DGKI	X										Diacylglycerol (DAG) activates Ras guanyl nucleotide-releasing proteins by activating the Ras signal. DAG kinase (DGKi) promotes the conversion of DAG to phosphatidic acid. DGKi methylation, in the context of methylated MGMT, would explain the poor response, having the Ras pathway activated.
CpG TRIM38									X	X	Negative regulator of innate immunity and inflammatory response
CpG TRIM58										X	Regulator of innate immune response
CpG MS4A7										X	Cell maturation in monocytes
IDH								X			Cycle of tricarboxylic acids; they catalyze the decarboxylation of isocitrate to alpha ketoglutarate. Their mutation generates depletion of NADPH, deoxynucleotides, and antioxidants, making these models more sensitive to radiotherapy.
SLC7A7	X							X			Amino acid transporter in the plasma membrane. It is overexpressed at the mRNA and protein levels and would confer proliferative capacity to the tumor cell.

Table 3 continues on next page

(CONT.) TABLE 3. **Summary of Molecular Processes With Statistical Significance That Interfere in Each of the Capacities Acquired and Necessary for Tumor Growth and Progression, Stratified According to Cancer Hallmarks**

Molecular process	Acquired capacities										Strategy
	1	2	3	4	5	6	7	8	9	10	
GLI1	X				X	X					Unlike in other types of cancer, here nGLI1 and PATCH at high levels correlated with better PFS.
PTCH	X				X	X					Unlike in other types of cancer, here nGLI1 and PATCH at high levels correlated with better PFS.
CMET	X		X		X	X					Involved with activation by synergistic network Ras, PI3K/Akt, SRC (proto-oncogene)
TCTN1	X				X	X					It would be a regulator of the Hedgehog downstream Gli-Smo pathway. Despite finding high levels of TCTN1 in glioblastoma, its role in the Hedgehog pathway is not clear.
PTEN			X								Loss of function of PTEN; releases survival signals of AKT
CD44	X					X					A cell surface antigen involved in cell migration and adhesion; stem cell marker
PP1A			X								It seems to be associated with p53 since it is overexpressed only in glioblastomas with mutated *TP53*.
P53			X								Genotoxic stress resulting from ionizing radiation or chemotherapeutics increases p53 levels, which induces the expression of proteins such as p21 or pro-proteins such as BAX and PUMA
CXCR4					X						A switch between the primary tumor and recurrence was observed, passing from VEFGR2-HIF1a to CXCL12-CXCR4 at both the mRNA and protein levels. This could imply a transition from angiogenesis to vasculogenesis.
HIF1A					X						The recruitment of myeloid precursors necessary for vasculogenesis would be mediated by CXCL12 and would be independent of HIF1a.
VEGFR2					X						Decrease in its expression with respect to the initial tumor, a product of the switch to vasculogenesis
LAPTM4B	X				X	X					Increase in angiogenesis. Activates the PI3K/Akt signal. It favors chemoresistance by increasing the release of chemotherapeutic drugs such as doxorubicin, paclitaxel, and cisplatin due to its relationship with the *MDR1* gene (multidrug resistance).
DNAPKS							X				The cytotoxicity of radiation therapy depends on its ability to generate double-stranded DNA damage. Nonhomologous recombination is the main mechanism to repair this damage and depends largely on the expression of DNA PKcs.
Cytochrome c oxidase			X					X			The increase in cytochrome oxidase increases the capacity for electron flow, more efficient mitochondrial coupling, and a decrease in the production of reactive ROS oxygen species, protecting the tumor cell.
Tetraspanin CD151	X				X	X					Activation of CDC42 and Rac: Rho family, motility. Activation of HGF/c-Met, PI3K/Akt/GSK-3b/Snail

CXCL12, CXC motif chemokine ligand 12; CXCR, chemokine receptor family; DGKI, diacylglycerol kinase iota; DNA-PKcs, DNA-dependent protein kinase; GLI1, glioma-associated oncogene homologue 1; HIF1a, hypoxia-inducible factor 1 subunit alpha; IDH, isocitrate dehydrogenase; IFIT1, interferon-induced protein with tetratricopeptide repeats 1; LAPTM4B-35, lysosomal protein transmembrane 4 beta; MGMT, O-6-methylguanine DNA methyltransferase; miR, microRNA; MS4A7, membrane-spanning 4-domains subfamily A member 7; NADPH, nicotinamide adenine dinucleotide phosphate; P53, protein 53; PFS, progression-free survival; PP1A, nuclear protein phosphatase 1 alpha; PTEN, phosphatase and tensin homologue; PTCH, patched; ROS, reactive oxygen species; SLC7A7, solute carrier family 7 member 7; TCTN1, tectonic family member 1; *TP53*, tumor protein 53; TRIM38, tripartite motif containing 38.

Acquired capacities and tumor progression stratified according to cancer hallmarks: (1) sustained proliferation signal, (2) evasion of suppressors, (3) evasion of apoptosis, (4) immortality, (5) angiogenesis, (6) invasion and metastasis, (7) genomic instability, (8) metabolism, (9) inflammation, (10) evasion of the immune system.

IHC, associating it with statistically significant differences in median survival: $P = .003$ (HR, 1.79; 95% CI, 1.15-2.8). Eoli et al[15] and Limam et al[9] did not find statistical significance by the same method. Marziali et al[10] and Weller et al[28] measured EGFRvIII without finding significant differences.

P53

Li et al[29] and Wang et al[17] used IHC to evaluate p53 expression or mutations in *TP53*, finding a significant difference (HR, 0.149) in median survival favoring patients with *TP53* mutation. Eoli et al[15] and Limam et al[9] did not find significant differences. Additionally, Limam et al[9] studied MDM2 measured by IHC without finding significant differences.

DISCUSSION

Although the clinical course of each patient with GB is unique and is influenced by the location of the tumor and their age, comorbidities, and Karnofsky Performance Status grade, among other factors, all patients receive the same treatment because no personalized treatment is yet available. Maximum safe resection is performed, in which the tumor is removed macroscopically. The patient subsequently undergoes radiotherapy and concomitant chemotherapy, if their comorbidities allow it, then receives only adjuvant chemotherapy for a generally short time, until the disease appears to be under control. However, relapse occurs in virtually 100% of patients because of the limited treatment options, few of which offer any evidence of increased survival. Cells resistant to multiple therapies persist in the brain parenchyma around the postoperative and postirradiated cavity. Genomic analysis pre- and post-treatment has shown that the recurrent tumor activates pathways associated with clonal and subclonal evolution; these are the origin of treatment failure, development of resistance, and, finally, tumor relapse.

Many molecular pathways and processes are activated during tumor relapse in GB, associated with its evolutionary process. These include: sustained proliferation signals, in which we identified the role of EGFR, miR, IFIT1, DGKI, SLC7A7, GLI1, and PTCH, among others; evasion of apoptosis, with different alterations in *TP53*, *PTEN*, *PP1A*, and *MGMT*; angiogenesis, associated with the differentiated expression of CXCR4, CXCL12, HIF1α, and VEGFR2; invasion processes, related to miR, tetraspanin CD151, LAPTM4B, and CD44 clusters; genomic instability, associated with alteration in repair mechanisms such as MGMT and DNA PKs (DNA-dependent protein kinase); energy dysregulation or metabolic changes associated with IDH; tumor-promoted inflammation in relation to miR and TRIM38 expression; and, finally, immune system evasion associated with TRIM58 or MS4A7 (Table 3). These processes, already described by Hanahan and Weinberg,[3] are not a series of steps accomplished one after another, but rather are launched at different times and in different ways in each tumor, reflecting their intratumoral and intertumoral heterogeneity.

The present review aimed to assess the clinical relevance of the markers identified in each of these different molecular processes or signaling pathways. Some markers have good evidence and clinical applicability, such as MGMT and IDH. Others have substantial theoretical evidence but discrepancies in clinical studies. Some markers that are good candidates emerge rapidly, such as miR and others; these constitute the vast majority of the evidence found, but they require more study in glioblastoma, although they have been described in other types of cancer. Notably, we also found biomarkers with good evidence of their involvement in tumor relapse expressed in terms of PFS, such as MGMT and IDH.

The *MGMT* gene is located on chromosome 10q26 and encodes a DNA repair protein that removes alkyl groups from the O6-position of guanine. Epigenetic silencing mediated by MGMT promoter methylation generates decreased DNA repair, causing greater chemosensitivity. Moreover, the absence of methylation means that each time an alkylating agent injures the guanine in the O6 position, it is repaired by MGMT, causing chemoresistance.[30] This methylation status appears in the multivariate analysis of 21 publications. Whenever a factor associated with recurrence is postulated, it must be verified that it is independent of the possible effect of the methylation status of MGMT. To our knowledge, MGMT methylation confers more chemosensitivity, but it remains uncertain why some tumors with methylation do not respond to initial therapy. Zhang et al[11] analyzed IFIT1 and found that MGMT inhibition is associated with its overexpression. Cohen et al,[31] when evaluating tumors with MGMT methylation without clinical response, found differential expression in DGKI, a RAS modulator.

IDHs are a group of enzymes fundamental in the tricarboxylic acid cycle. They catalyze the decarboxylation of isocitrate to α-ketoglutarate. IDH1 acts at the level of cytosol and peroxisome, while IDH2/3 acts at the mitochondrial level. IDH1/2 has important functions associated with glucose consumption, lipogenesis, glutathione catabolism, and defense against reactive oxygen species and radiation.[32] The *IDH1* mutation identified by De Carlo et al[24] and Wang et al[17] is found more frequently in secondary glioblastomas and generates depletion of NADPH, deoxynucleotides, and antioxidants, making these models more sensitive to radiotherapy and more likely to be associated with a higher PFS. Tumors with nonmutated *IDH1*, according to Lalezari et al[6] and Etcheverry et al,[25] are

more frequently primary glioblastomas and associated with a lower PFS.

Under normal conditions, in response to genotoxic stress, p53 protein induces cycle stop, mainly in the G1 phase to repair the DNA damage; ultimately, this leads to apoptotic cell death or senescence, thus preventing cell replication with DNA damage. Genotoxic stress secondary to ionizing radiation or chemotherapeutics increases p53 levels, which induces the expression of regulatory cell cycle proteins, such as p21, or pro-apoptotic proteins, such as BAX and PUMA.[29] *TP53* mutation, studied by Li et al,[29] Wang et al,[21] Eoli et al,[19] and Limam et al,[9] is associated with chemosensitivity and higher PFS, while functional p53 is associated with a lower PFS.

Some of the most attractive markers currently under research are miRs: small noncoding RNA molecules, highly conserved, and composed of between 18 and 25 nucleotides. They are responsible for the posttranscriptional negative regulation of gene expression. Bioinformatics tools estimate that miRNAs regulate up to 60% of human genes, including genes associated with chemo- and radioresistance. miRNAs generally act in clusters and adopt the role of oncogenes or tumor suppressor genes, depending on their target. In this way, they can inactivate suppressor genes or activate oncogenes.[20]

Wang et al,[8] when comparing the miRNA profile of glioblastomas with that of healthy subjects in peripheral blood, has found a decrease in the expression of miR-485-3p, suggesting its role as tumor suppressor. Its participation in chemoresistance has also been described. This decrease in expression was associated with lower disease-free survival. Cheng et al[26] studied miR 222, which is widely recognized as an oncogene. The miRNAs showed differential expression between tumors with and without *MGMT* methylation. Therefore, the signature constituted by miR-222, miR-145, miR-20a, miR-132, and miR-129 was postulated by Cheng et al[26] as associated with a decrease in PFS even in the presence of promoter methylation.

From our perspective, there is ever-increasing knowledge of gliomagenesis and why successful treatment has been so elusive. We have synthesized a great deal of evidence and framed it in the landmarks of cancer proposed by Hanahan and Weinberg. However, the new therapies have only slightly increased PFS, with no benefit to OS, which shows that we still have "lost pieces." As such, it is necessary to standardize laboratory tests as well as the exhaustive application of current criteria for the follow-up of patients at the time of evaluating a tumor recurrence. The new therapies have expanded our vocabulary with terms like pseudoprogression and pseudoresponse. We believe that the best way to evaluate these "escape routes" is by analyzing recurrence in terms of the central precepts of molecular biology, and what occurs at many levels: DNA, RNA expression, transcriptional, protein and posttranscriptional, and epigenomic. We have ever-more alternatives framed in immunotherapy, vaccines, target therapies, and bioprospecting studies. Multidisciplinary research is clearly necessary—including basic, clinical, and bioengineering research—which at some point must achieve longer life expectancies for our patients.

Limitations

A limitation of the review is the large amount of isolated data presented, which is useful for research but not as relevant in the clinical field if no other studies exist to support them. Moreover, many studies lack information on PFS, and for this reason 21 studies initially considered were excluded. Information about disease-free survival was requested from all authors via email, but few data were obtained. Finally, the categorization of molecular processes according to the evolutionary processes described is difficult to achieve due to the great integration of the pathways. Occasionally it becomes a somewhat subjective task, although it is very useful to visualize tumor escape pathways.

Another limitation is the lack of standardization in the tests; results may vary according to the proposed analysis. With MGMT, for instance, results differ depending on the technique used. Likewise, there is a lack of standardization for the use of radiological criteria of relapse; some studies still use the McDonald criteria, while others use the RANO or the iRANO criteria.

CONCLUSIONS

It is possible to identify a wide variety of signaling pathways and molecular processes implied in the relapse of GB, as well as in the evolution of many types of cancer. This diversity would explain intra- and intertumor heterogeneity, treatment evasion, and, finally, relapse. However, there are few molecular processes for which robust evidence is available that have resulted in clinical utility. Therefore, it is necessary to subject the candidate processes to additional clinical trials, to build a larger body of evidence that allows personalization of GB therapy. In this context, knowledge of the signaling pathways activated in each case will determine the type of treatment and the temporal pattern of its administration. ■

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