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DNA Double-Strand Breaks Repair and Signaling of Human Gliomas and Normal Brain Cells in Response to Radiation: Potential Impact of the ATM- and BRCA1- Dependent Pathways

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1. Introduction

Deriving from glial, astrocyte, or dendrocyte cells, gliomas are the most frequent tumors of central nervous system. Unfortunately, most of gliomas are refractory to standard radiotherapy treatments. The median survival for patients bearing grade IV gliomas (glioblastomas) does not exceed one year even after both aggressive surgery and radiotherapy treatment (Behin et al., 2003). A standard of 60 Gy delivered in 30 fractions during six weeks remains the best radiotherapy modality against gliomas (Behin et al., 2003). This last conclusion raises the possibility that human gliomas might be generally more radioresistant than other tumor tissues. However, there is no consensus in literature about a specific radioresistance of human gliomas. Besides, the complexity of the molecular and cellular features of radiation response and the difficulty to define reliable endpoints to account for radiosensitivity whatever the tissue type may have limited the extent of some reports. In addition, three specific features of radiobiology of gliomas can be also evoked:

- cellular *in vitro* endpoints like clonogenic survival seem to be less appropriate to predict gliomas radiocurability than that of other tumour types (Taghian et al., 1992, 1993);
- animal models extensively used in glioma research may not reflect specificities of human gliomas and may bias in anti-glioma strategies (Holland, 2001);
- DNA repair capacity of gliomas is poorly documented and most of the investigations about genes mutations concern actors of proliferation rather than upstream DNA repair proteins (Zhu & Parada, 2002).

In 2004, our group obtained the most protracted survival of rats bearing radioresistant rodent gliomas by using synchrotron X-rays combined with *intracerebral* cisplatin injection. Such so-called PAT-plat treatment triggers the photoactivation of platinum atoms and produces some additional DNA double-strand breaks (DSBs) at the vicinity of cisplatin-induced DNA adducts (Biston et al., 2004). The severity of PAT-Plat-induced DSBs was shown to be due to the inhibition of the major DSB repair pathway in mammalians, namely

the non-homologous end-joining (NHEJ) process (Biston et al., 2004; Corde et al., 2003). NHEJ is required for cell viability and its inhibition has been shown to be systematically linked to radiosensitivity. This DSB repair pathway is dependent on the ATM kinase activity that may be considered as the major DNA-breaking stress signaling pathway in mammalians. The inhibition of NHEJ may be rescued by other repair pathways, like the RAD51- and/or MRE11-dependent recombination process (Dudas and Chovanec, 2004; Joubert et al., 2008). Interestingly, these last two recombination pathways were shown to require a functional BRCA1 protein, a tumor suppressor whose mutations are responsible for inherited breast cancers (Scully et al., 1997). BRCA1 is a phosphorylation substrate of ATM kinase and also required for a normal response to radiation and to alkylating agents. Consequently, tumors showing impaired BRCA1 were supposed to be sensitive to the PAT-Plat effect described above and to a number of chemotherapy drugs like cisplatin (Bhattacharyya et al., 2000; Corde et al., 2003).

In the particular case of preclinical anti-glioma radio-chemotherapy trials, the most extensively used modalities are based on syngenic rat models subjected to intracranial inoculation of non-imunogenic cell lines. This is notably the case of the C6, 9L, F98 rodent glioma cell lines. However, that all these rat glioma models are induced by N-nitrosmethylurea (NMU) or Ethylnitrosourea (ENU) -mediated mutagenesis ; which may condition their response to stress (Table 1). Since the choice of rodent glioma models is mainly motivated to date by the existence of previous raw data in the lab, their proliferation capacity in culture and/or in animals and their non-immunogenic properties, it may introduce some biases in data interpretation.

Cell line	Tumour type	Mutagen	P53 status	p16/CDKn2a/ Ink4	BRCA1 localisation after X-rays	Depositor
C6	Gliosarcoma	MNU, repetitive dose	Wild type	*Mutated*	Cytoplasm	(Benda et al., 1968)
9L	Gliosarcoma	MNU, repetitive dose	*Mutated*	*Wild type*	Nuclear	(Benda et al., 1968)
F98	Anaplastic glioma	ENU, single dose	Mutated	*Mutated*	Weak	(Wechsler et al., 1972)

Table 1. Origin and biological features of the major rodent glioma cell lines

In 2008, to evaluate the impact of the choice of a rat glioma models in a study dealing with radio- or chemotherapy, cell death pathways, cell cycle arrests, DNA repair and stress signalling were examined in response to radiation and cisplatin in C6, 9L and F98 models (Bencokova et al., 2008). Rodent glioma models showed a large spectrum of cellular radiation response. Surprisingly, BRCA1 was found to be functionally impaired in C6 and F98 favouring genomic instability, tumour heterogeneity and tolerance of unrepaired DNA damage. Furthermore, since BRCA1 acts as a tumor suppressor in a number of malignancies, our findings raise also the question of the implication of BRCA1 in brain tumors formation (Bencokova et al., 2008).

The importance of DSB repair and signaling proteins in the radiation response prompted us to investigate the radiobiological features of a number of human brain tumors, notably the activity of the NHEJ and the BRCA1-dependent pathways, in order to propose molecular assays to predict the response of gliomas to anti-cancer treatments. Some recent conceptual and technical advances in the DSB repair field have motivated such approach: 1) the importance of the potential interplay existing between the two major DSB repair pathways, NHEJ and recombination that may condition the final response to radiation (Joubert et al., 2008); 2) the existence of a temporal hierarchy between ATM- and BRCA1-dependent phosphorylation events occurring after irradiation and conditioning cell cycle arrests and cell death pathways (Foray et al., 2003); 3) the fact that immunofluorescence permits to date the quantification of the DSB induced by radiation inside each cell nucleus via biomarkers that are specific to one particular step of DSB repair and signaling. This is notably the case of γ-H2AX that is the sensor of DSB managed by NHEJ and may serve as a marker of NHEJ activity (Joubert et al., 2008; Rothkamm & Lobrich, 2003). In 2011, by having accumulated a number of immunofluorescence data in response to 2 Gy X-rays in about 200 human normal and tumor cell lines, we propose a molecular model of radiation response in which the nucleo-shuttling of active ATM forms stimulates NHEJ and inhibits exacerbated nuclease activity of MRE11 responsible for genomic instability. BRCA1 is one of the major ATM phosphorylation substrate involved in this model (Granzotto et al., 2011, submitted) (Fig. 1). Hence, in this work, we have systematically examined the γ-H2AX, ATM and BRCA1 response of 13 human glioma cell lines and 6 normal brain tissues to X-rays.
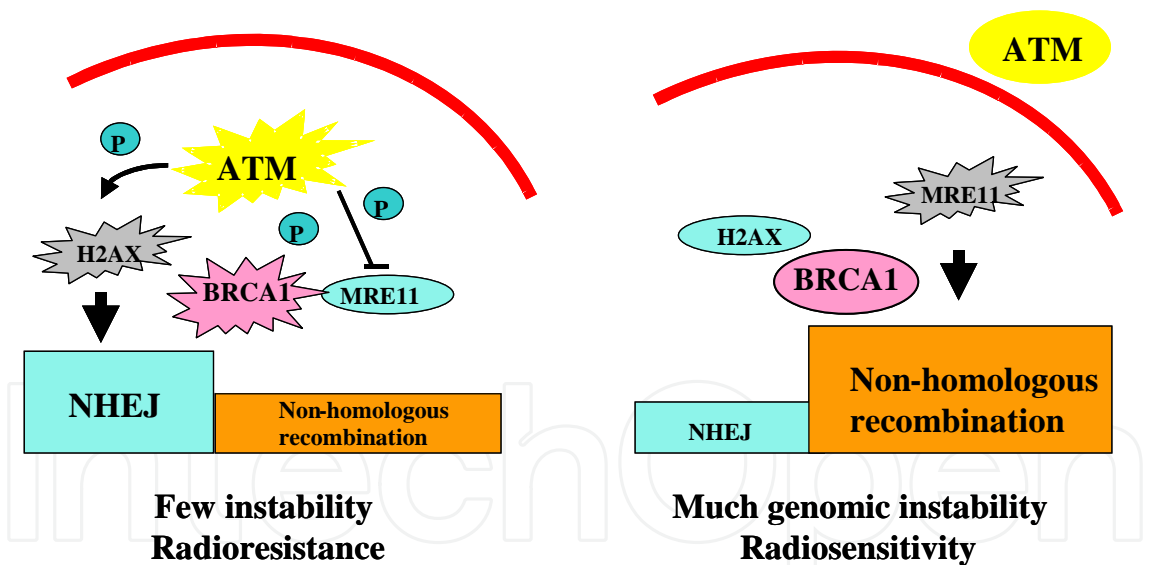

Fig. 1. Model for describing the radiation-induced ATM nucleo-shuttling

2. Materials and methods

2.1 Cell lines

Human glioma cell lines detailed in Table 2 are provided from adult donors and are purchased by commercial collections such as ATCC. They were routinely cultured as monolayers with Dulbecco's modified Eagle's minimum medium (DMEM) (Gibco-Invitrogen-France, Cergy-Pontoise, France), supplemented with 20% fetal calf serum and antibiotics.

Cell line	Tumour type	Donor age	BRCA1 functional status
SW1783 (HTB 13)	Glioblastoma astrocytoma	68	nd
U87MG (HTB 14)	Glioblastoma astrocytoma	44	+
U118MG (HTB 15)	Glioblastoma astrocytoma	50	-
U138MG (HTB 16)	Glioblastoma astrocytoma	47	-
U373MG (HTB17)	Glioblastoma astrocytoma	nd	+/-
LN229	Glioblastoma PTEN$^{+/+}$	60	+/-
CCF-STTG1	Astrocytoma	68	-
MO59J	Glioblastoma	33	+
U251	Glioblastoma PTEN $^{-/-}$	nd	nd
T98G	Glioblastoma multiforme	61	-
GHD	Glioblastoma	nd	nd
CB193	Glioblastoma	nd	-
SF767	Glioblastoma multiforme	nd	nd

Table 2. Human glioma cell lines used in this study and their BRCA1 functional status

Normal human brain cells detailed in Table 3 are provided from fetal brain and purchased by Sciencell Research Laboratories (Carlsbad, CA, USA). They were routinely cultured as monolayers with medium, serum and growth complement recommended by Science Research Laboratories.

Cell line	Cell type and brain localization	Sciencell Reference
HA	Astrocytes - cortex	#1800
HAc	Astrocytes - cerebellum	#1810
Hsc	Schwann cells	#1700
Hah	Astrocytes - hippocampal	#1830
HMC	Meningeal cells	#1400
Hasp	Astrocytes – spinal cord	#1820

Table 3. Human normal cell lines used in this study and their origin

2.2 Irradiation

An orthovoltage X-ray clinical irradiator was used to perform all the irradiations. It is described elsewhere (Joubert et al., 2005). The dose-rate was approximately 1.234 Gy.min^{-1}.

All the experiments were performed on cells at plateau phase of growth to avoid any bias due to cell cycle.

2.3 Immunoblottings

Preparation of nuclear extracts, immunoblotting were performed using protocols published elsewhere (Foray et al., 2003). Anti-*pBRCA1*[ser1423] (Upstate Biotechnology-Euromedex) and anti-*BRCA1* antibodies (Santa Cruz, CA, USA) were used at 1:1000 dilution.

2.4 Immunofluorescence

Immunofluorescence protocol was described elsewhere (Foray et al., 2003). Cells were fixed in paraformaldehyde and permeabilized for 5 min at 4°C. Anti-*γ-H2AX*[ser139] antibody was purchased from Upstate Biotechnology-Euromedex, Mundolsheim, France) and used at 1:800. Anti-*pATM*[ser1981] (Abcam, Cambridge, UK), anti-*pBRCA1*[ser1423] (Upstate Biotechnology-Euromedex) and anti-*BRCA1* antibodies (Santa Cruz, Tebu-Bio, Le Perray, France) were used at 1:100 dilution. Incubations with anti-mouse TRITC or with anti-rabbit FITC secondary antibodies (Sigma-Aldrich) (dilution at 1:100) were performed at 37°C in 2% BSA for 20 min. Nuclei were counterstained by 4,6-Diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) for 10 min. Coverslips were mounted in Vectashield (Abcys, Paris, France) and examined with an Olympus fluorescence microscope. Fifty nuclei per condition were analyzed. DAPI staining permitted also to indirectly evaluate yield of G_1 cells (nuclei with homogeneous DAPI staining), S cells (nuclei showing numerous γ-H2AX foci), G_2 cells (nuclei with heterogeneous DAPI staining) and metaphase (visible chromosomes) (Joubert et al., 2008). It is noteworthy that in our conditions, the ATM and DNA-PK kinases that phosphorylate the H2AX histones require some minutes post-irradiation to be fully active. Consequently, the earliest time post-irradiation was fixed at 10 min, in agreement with previous reports (Joubert et al., 2008).   The quantification of foci was performed by eye-scoring and verified by semi-automatic scoring Cell[F] software from Soft Imaging System GmbH (Münster, Germany) that permits to quantify nuclei and foci size.

3. Results

3.1 Glioma cell lines elicit a wide range of radiation-induced DSB repair capacity

In a previous report, we have shown that the number of unrepaired DSB assessed 24 h after 2 Gy is quantitatively correlated with intrinsic radiosensitivity reflected by clonogenic cell survival at 2 Gy (SF2) (Joubert et al., 2008). As a first step, we focused therefore on the DSB repair capacity of the human gliomas by using *anti- γ-*H2AX immunofluorescence. Glioma models described a continuous range of responses between 2 to 58% of unrepaired DSBs, corresponding to an SF2 range of 80 to 2%, respectively (Fig. 2). In agreement with previous reports, MO59J cells exhibited one of the most severe human DSB repair deficiency (Chavaudra et al., 2004; Lees-Miller et al., 1995) (Fig. 2).

By taking into account survival data available in literature (Joiner et al., 2001), our findings were in agreement with a general correlation between SF2 and unrepaired DSBs obtained from 20 human tumour cell lines (Chavaudra et al., 2004). Here, the human glioma cell lines tested describe therefore a wide range of NHEJ repair capacity and radiosensitivity that does not suggest a global tendency to radioresistance for brain tumor cells. Interestingly, the average DSB repair rate of human radioresistant skin fibroblasts appears to be systematically faster than that of the glioma cell lines tested here.
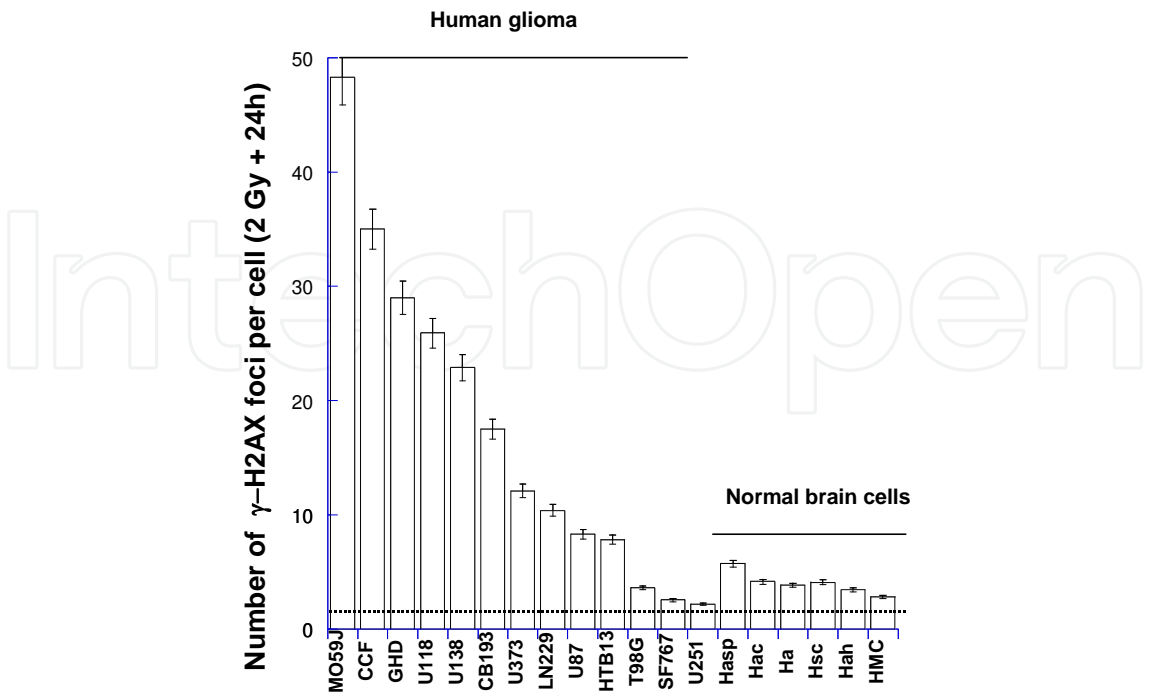
Fig. 2. Number of γ-H2AX foci assessed 24 h after 2 Gy X-rays in the indicated human tumour and normal brain cell lines. Data shown are the mean ± standard error of 3 independent replicates, at least. Dotted cell lines indicated the average corresponding value of γ-H2AX foci for radioresistant skin fibroblasts published elsewhere (Joubert et al., 2008).

3.2 Most glioma cell lines elicit impaired radiation-induced ATM kinase activity

Since phosphorylation of γ-H2AX requires the ATM kinase activity, cell lines were also subjected to immunofluorescence with antibodies against the autophosphorylated forms of ATM (pATM) (Bakkenist and Kastan, 2003). Very few pATM foci were observed constitutively, suggesting a moderate ATM kinase activity in all the glioma models (data not shown). MO59J elicited a marked delay in the radiation-induced ATM activation, likely due to its ATM mutations (Tsuchida et al., 2002). Although the other cell lines exhibited a various number of pATM foci at 10 min post-irradiation ranging from about 1 to 70 pATM foci per cell, the great majority of them show a ATM kinase activity at 10 min post-irradiation that is abnormally lower than radioresistant skin fibroblasts (Fig. 3).

3.3 Most glioma cells elicit impaired radiation-induced BRCA1 phosphorylation

Ionizing radiation cause BRCA1 phosphorylation, visible by a protein migration shift (Scully et al., 1997a,b). Among 13 glioma cell lines of the collection, 8 were subjected to anti-*BRCA1* immunoblots. Our results revealed that BRCA1 migrated at the expected size for all the cell lines. However, BRCA1 appeared differentially expressed: U138 and U373 elicited the weakest spontaneous BRCA1 expression and U87, MO59J and GHD the highest one (Fig. 4). Only 3 (MO59J, U87, GHD) among 8 irradiated cell lines showed the typical shift of BRCA1 phosphorylation, suggesting that the functional BRCA1 status is impaired in the great majority of glioma cell lines. U373 and LN229 showed a second band above BRCA1 that seemed too high to represent any BRCA1 phosphorylation signal (Fig. 4).
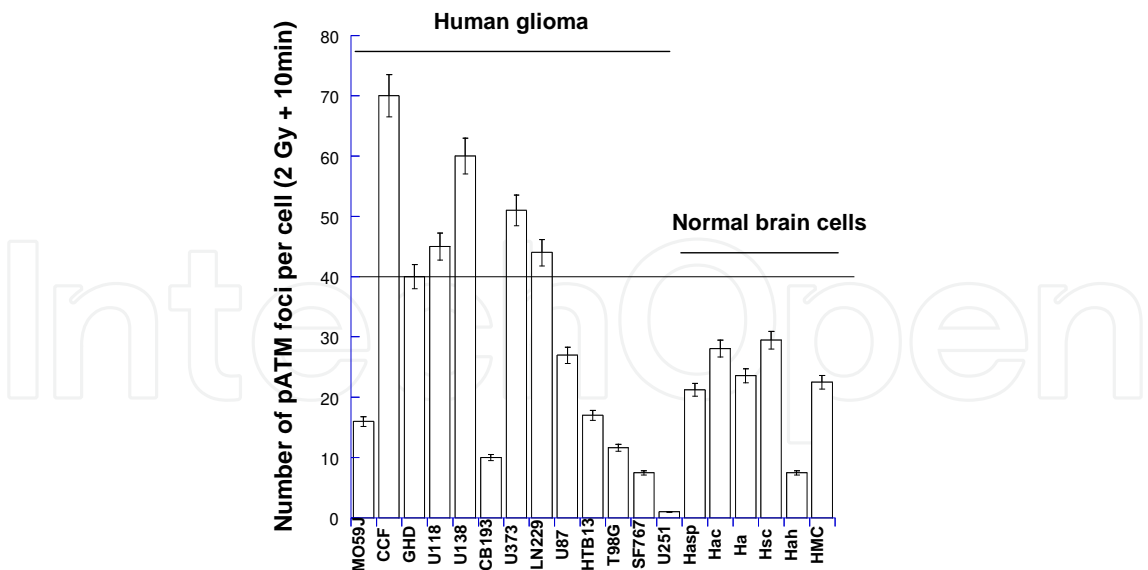
Fig. 3. Number of pATM foci assessed 10 min after 2 Gy X-rays in the indicated human tumour and normal brain cell lines. Data shown are the mean ± standard error of 3 independent replicates, at least. Dotted cell lines indicated the average corresponding value of pATM foci for radioresistant skin fibroblasts obtained in our lab (Granzotto et al., submitted).
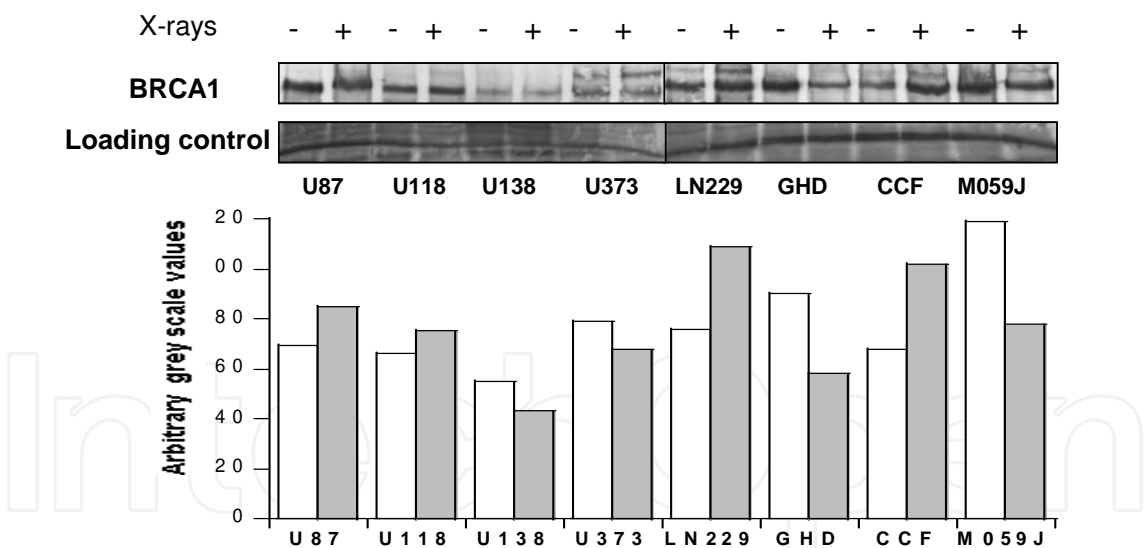

Fig. 4. Representative example of anti-*BRCA1* immunoblots of nuclear extracts from the indicated human cells exposed to 15 Gy followed by 4 h for repair. Expression of BRCA1 was quantified by grey scale analysis in arbitrary units.

In agreement with anti-*BRCA1* immunoblots, only MO59J, U87, GHD elicited radiation-induced BRCA1 foci. It is however noteworthy that some cell lines exhibit spontaneous BRCA1 foci likely due to S-G2/M phase, consistent with the findings that radiation and cell cycle triggers the phosphorylation of different BRCA1 serine residues (Bakkenist & Kastan, 2003; Gatei et al., 2000; Xu et al., 2001) (Fig. 5A). Immunofluorescence with antibodies

against the phosphorylated BRCA1 Ser^{1423} (pBRCA1^{ser1423}) was performed, as well. Again, only MO59J, U87, GHD exhibited positive pBRCA1^{ser1423} signals (Fig. 5B). Altogether, these findings suggest that BRCA1 is functionally impaired in the majority (5/8) of human glioma models in response to radiation. It must be reminded that functional BRCA1 impairments are due either to *BRCA1* mutations or to the absence of BRCA1 protein partners, like RAD51 (Scully et al., 1997a,b). Particularly, all the cell lines exhibited RAD51 foci whether spontaneously (in S/G2M) after irradiation (data not shown). Hence, BRCA1 impairments were observed in the majority of human glioma models, independently of their RAD51 activity.
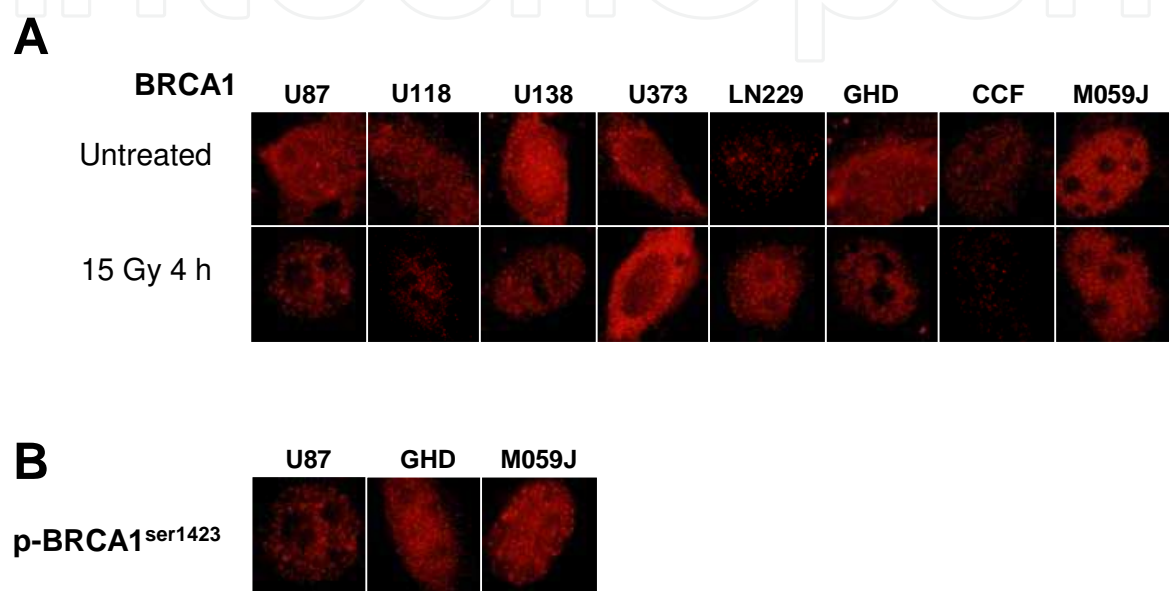
A

B

Fig. 5. A. Anti-*BRCA1* immunofluorescence applied to the indicated cell lines after 15 Gy X-rays followed by 4 h for repair. B. Representative examples of pBRCA1^{ser1423} signals obtained in U87, GHD and M059J cell lines in the same conditions.

3.4 Normal brain cells elicit a wide range of radiation-induced DSB repair capacity

Human brain cells showed few residual γ-H2AX foci after 2 Gy followed by 24 h for repair, suggesting a larger radioresistance than the human glioma cell lines tested (Fig.2). However, inside the normal brain cells group, there is no significant difference between the brain localizations tested (Fig.2). Conversely, the yields of γ-H2AX foci of human astrocytes are systematically higher than radioresistant fibroblasts (p<0.02) (Fig. 2).

3.5 Normal brain cells elicit impaired radiation-induced ATM kinase activity

In order to investigate further the radiobiological features of human normal brain cells, we assessed the number of pATM foci 10 min post-irradiation. Normal human brain cells exhibited significantly less γ-H2AX foci 10 min post-irradiation than radioresistant fibroblasts (p=0.01), suggesting that ATM-dependent DSB recognition is slightly impaired in normal brain cells (Fig. 3). The measured ATM kinase activity in normal human brain cells lead to a lack of recognition of about 10 to 30 DSB at the dose of 2 Gy, corresponding to about 12.5 to 37.5% of radiation-induced DSB, respectively. Again, no significant difference was observed between the brain localizations tested.

3.6 Normal brain cells elicit impaired radiation-induced BRCA1 phosphorylation

In our attempts to investigate the functional status of BRCA1 in response to radiation, we have systematically observed a very weak BRCA1 and pBRCA1 signals in all the normal brain cells, whatever the conditions, suggesting a significant impairment of the functional status of this protein (data not shown). It is noteworthy that even in the rare normal brain cells observed in S or G2/M phase, the yield of BRCA1 foci was negligible.

3.7 Intercomparisons between human glioma and normal brain cells

In order to compare glioma and normal brain cells data, we plotted, for all the cell lines tested, the number of γ-H2AX foci per cell assessed 24 h post-irradiation (that is an indicator of radiosensitivity) against the number of pATM foci assessed 10 min post-irradiation (that is an indicator of the early ATM kinase activity in response to radiation) (Fig. 6).
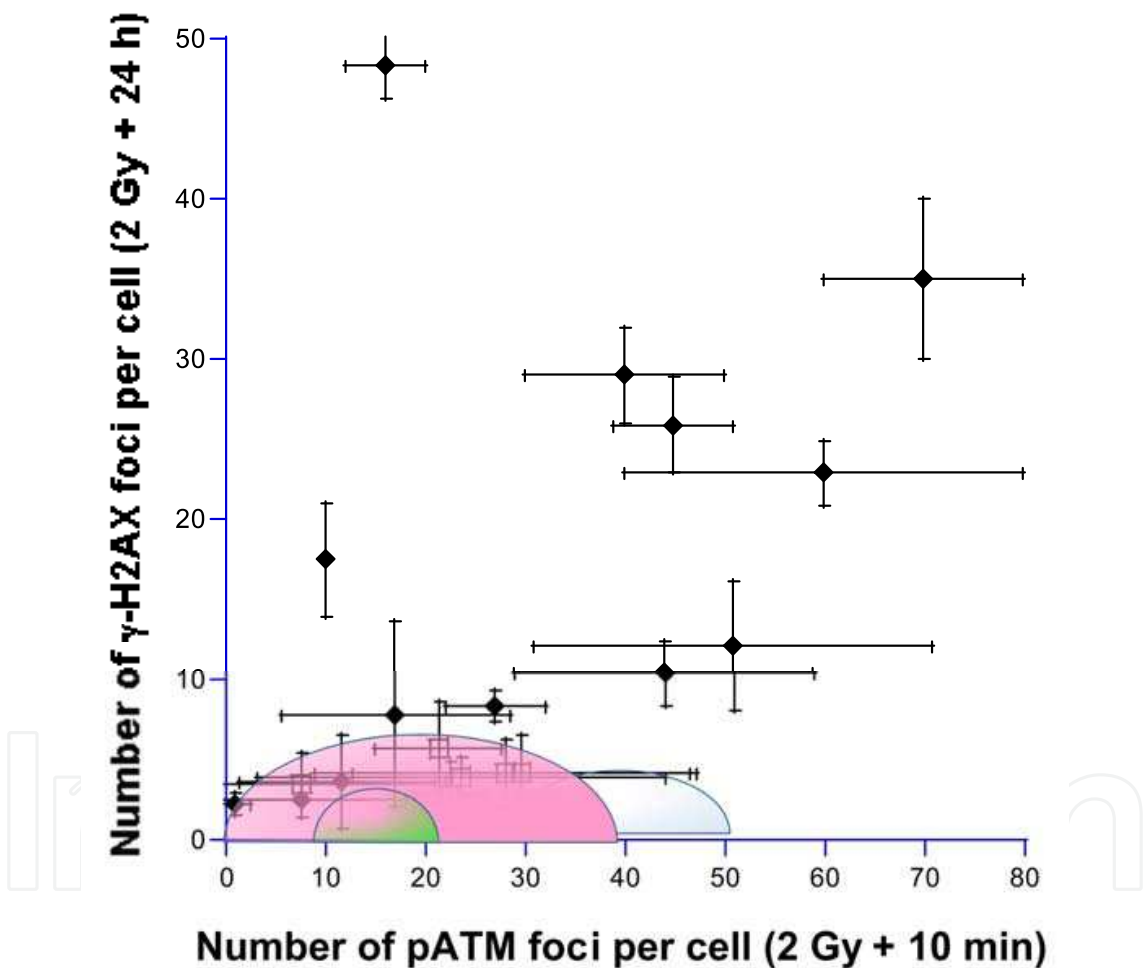

Fig. 6. Number of residual γ-H2AX foci per cell as a function of the corresponding number of pATM foci per cell for all the human glioma (closed losanges) and normal brain cells (open squares) described in this report. All the foci data are shown in Fig. 1 and 2 as histogram. Each plot is represented by the mean ± standard error of 3 independent replicates, at least. Blue and pink confidence zones correspond to the values from human primary fibroblasts belonging to the radiosensitivity group I and II, respectively (see Discussion). Green confidence zone corresponds to values from the C6, 9L and F98 rodent glioma cells.

The fig. 6 clearly shows that the normal brain cells tested here are more DSB repair deficient with a lower ATM kinase activity than the most radioresistant skin fibroblasts. Conversely, the normal brain cells may belong to a group of cells characterized by a moderate but significant radiosensitivity associated with genomic instability. With regard to the glioma cells, they covered all the range of DSB repair and ATM kinase activity, likely due to their intrinsic instability. Interestingly, the γ-H2AX and pATM values from the C6, 9L and F98 rodent glioma cells correspond to a very restricted genetic conditions that are not representative of human glioma.

4. Discussion

4.1 New advances in DSB repair models

As evoked in the Introduction chapter, it has been demonstrated that radiation-induced DSB can be determined from the number of nuclear foci formed by the phosphorylation of the variant histone H2AX and easily quantifiable using immunofluorescence (Rothkamm & Lobrich, 2003). Although successfully tested in hyper-radiosensitive cells and presented as powerful predictive assay, some preliminary data showed us that *γ-H*2AX immunofluorescence does not necessarily predict the whole range of human radiosensitivity. Recently, unlike the majority of studies focusing on mutations of one single gene, we have deliberately chosen to extend our investigations to the largest spectrum of radiosensitivity possible with human cells, independently of gene mutations. The relationship between cellular radiosensitivity and DSB repair data was examined in a collection of 40 non-transformed human fibroblasts representing at least 8 different genetic syndromes (Joubert et al., 2008). The systematic application of the most extensively used molecular assays, namely immunofluorescence, PFGE and plasmid assays allowed us to propose a quantitative correlation between molecular and cellular radiosensitivity that is relevant for all mammalian cells: survival fraction at 2 Gy (SF2) was found to be inversely proportional to the amount of unrepaired DSB, whatever the genes mutations and the assays applied.

Form this correlation, a classification of genetic diseases associated with cellular radiosensitivity in 3 groups was also proposed in our recent study (Joubert et al., 2008): group I: radioresistance; group II: moderate radiosensitivity and high cancer proneness; group III: hyper-radiosensitivity. Obviously, the definition of these three groups tentatively proposed is provisional and is conditioned to the extension of additional cell lines in our systematic study of human radiosensitivity. A number of other biomarkers and tissue types have to be investigated to better document the molecular and cellular bases of this classification: this is notably the case of ATM, BRCA1, DNA-PK, 53BP1 and NBS1 proteins, all involved in the radiation response and of brain, breast, prostate tissues, all involved in the most frequent cancers.

Our results show that human glioma may exhibit a very wide range of radiosensitivity and may be characterized by impaired radiation-induced ATM kinase activity and BRCA1 phosphorylation. Normal brain cells share with gliomas these last two features. Conversely, can these results suggest that impaired ATM kinase activity and BRCA1 phosphorylation are specific to brain cells, whether normal or not?

4.2 Role of ATM in the radiation response of the brain

Ataxia telangiectasia (AT) is caused by homozygous ATM mutations and is a rare autosomal dominant syndrome associated with the highest radiosensitivity in humans (McKinnon,

1987). AT is a neurodegenerative disease. The prominent neurological sign of AT is an inexorable loss of cerebellar function, cerebellar atrophy, especially in vermal regions and loss of Purkinje cells (Lavin, 2008; McKinnon, 1987; Taylor et al., 1994). The role of ATM in brain cells and the dramatic consequences of its deletion or inhibition is well-documented but studies mianly concern rodent models. For example, it has been shown recently that cell populations in the Atm-/- central nervous system may be radioresistant (Gosink et al., 1999). To define specific radiosensitivities of neural populations, Gosink et al. (1999) have analyzed Atm-/- astrocytes and showed that Atm-/- astrocytes exhibit premature senescence, express constitutively high levels of p21, and have impaired p53 stabilization. However, in contrast to radiosensitive Atm-/- fibroblasts and radioresistant Atm-/- neurons, radiosensitivity of Atm-/- astrocytes was similar to wild-type astrocytes (Gosink et al., 1999).

By contrast, studies about the role of ATM on normal brain cells are more rare but also dominated by rodent models. For example, Soares et al. (1998) have shown that Atm expression during mouse development was highest in the embryonic mouse nervous system, where it was predominantly associated with regions undergoing mitosis. During the period of Purkinje cell neurogenesis, Atm was highly expressed in the area containing Purkinje cell precursors. However, in the postnatal cerebellum, Atm expression in Purkinje cells was very low, while expression in proliferating granule neurons was high. The only region of the adult nervous system that exhibited elevated Atm expression were the postmitotic sensory neurons of the dorsal root ganglia (Soares et al., 1998). Their data suggest an early developmental requirement for ATM in the cerebellum, and other regions of the central nervous system, but a global decrease of ATM expression.

Here, our data suggest that cytoplasmic ATM kinase activity in human brain cells is endogenously lower than in fibroblasts. According to our molecular model described in Fig. 1, normal brain cells may be a bit more radiosensitive to primary fibroblasts but overall more proned to radiation-induced genomic instability. Hence, while human glioma cells may exhibit a wide range of X-rays response, the fact that normal brain cells surrounding tumors may be radiosensitive should lead to cautiousness about the clinical transfer of anti-glioma radiotherapies.

4.3 Response of brain to radiation and gliomagenesis : Importance of BRCA1

BRCA1 is a phosphorylation substrate of ATM. Hence, if ATM kinase is naturally less active in normal brain cells, it is logical to observe impaired BRCA1 phosphorylation in normal brain cells. BRCA1 is an interesting protein in carcinogenesis because of its involvement in both breast cancers and DNA repair (Foray et al., 1999; Huen et al., 2010). Furthermore, its role is very important in chemotherapy since this protein is required for a normal response to alkylating agents (Bhattacharyya et al., 2000; Corde et al., 2003). However, up to date, nothing has really related BRCA1 to brain tumors. In 2008, our systematic radiobiological analysis of pointed out the possibility that BRCA1 may be not functional in rodent glioma models whose majority was induced by nitrosourea treatment. Interestingly, a paper published in 2003 presented ENU mutagenesis as an interesting tool to produce knockout rats, and especially BRCA1 and BRCA2 mutants (Zan et al., 2003). Hence, BRCA1 impairments in rodent glioma models may not have the same origin than those observed in human gliomas. This remark raises therefore the question of the relevance of the use of chemo-induced tumours in preclinical trials. This question is inasmuch important as Fig.6 shows that radiobiological features of rodent glioma models are not representative of those of human gliomas.

There were considerable advances in the investigations on gliomas-specific molecular markers. However, the great majority of them generally focus on actors of proliferation. A number of loss of heterozygosity have been reported in gliomas but there is still no consensus for any specific molecular signature (Bredel et al., 2005; Zhu & Parada, 2002). Very few concerned p53 and BRCA1. Loss of p53 and activation of growth factor receptor tyrosine kinase signalling pathway initiates tumour formation whereas disruption of RB pathway contributes to the progression of tumour development (Bredel et al., 2005; Zhu & Parada, 2002). However, the great majority of targeted proteins that act in the proliferation process, are partners of BRCA1 but are mainly *gatekeepers* than *caretakers* like BRCA1. This is notably the case of the p16/Cdkn2a/Ink4a whose homozygous deletions have been observed in C6 and F98 but not in 9L rodent glioma models (Bencokova et al., 2008). The p16/Cdkn2a/Ink4a protein belongs to the BRCA1-dependent cascade of stress-induced events, was shown to co-precipitate with BRCA1, and phosphorylate it at serine 1497 in response to cell cycle progression and DNA damage (Ruffner et al., 1999). Recently, it appears that BRCA1-negative cells show high p16/Cdkn2a/Ink4a cyclin-dependent kinase activity and that are 2- to 4-fold more sensitive to CDK inhibitors (Deans et al., 2004). Hence, the p16/Cdkn2a/Ink4a deletions found in C6 and F98 but not in 9L, together with our findings that BRCA1 is functionally impaired in C6 and F98 but not in 9L, suggest a model in which ATM- and p16/Cdkn2a/Ink4a-dependent may interplay with BRCA1.

To date, two major models of glioblastomas formation are proposed: glioma generation would be mediated either by genomic instability and uncontrolled differentiation or by rapid transformation of some pre-existing neural stem cells (Zhu & Parada, 2002). BRCA1 impairments are rather consistent with a glioma generation facilitated by genomic instability. Impaired BRCA1 may notably contribute to the lack of control of tyrosine kinase pathways that exacerbate cellular proliferation (Foray et al., 2002). Our findings raised the question of the implication of this protein in the tumorigenicity of brain tumours as well. Such assumption is supported by the fact that BRCA1 tumor suppressor activity is not necessarily restricted to inherited breast and/or ovarian cancer (Rosen et al., 2005). Notably, adenovirus experiments pointed out the potential role of BRCA1 in lung and colon malignancies (Marot et al., 2006). Our data suggest therefore that, in addition to an endogenously low ATM kinase activity that would be specific to brain cells and may lead to a reduced BRCA1 function, gliomagenesis may be facilitated by mutations in proteins partners of a complex including BRCA1.

5. Conclusions

Radiobiological investigations on extensively used rodent models have revealed that nitrosourea-directed mutagenesis may select particular mutations of BRCA1 genes that can be at the origin of the glioma formation. This BRCA1 mutation and impairment has some consequences on the radiation response but overall on the chemo-response of rodent glioma. Hence, rodent glioma models may not be representative of the human glioma models. Once the role of BRCA1 has been pointed out, the observation that radiation-induced phosphorylation of BRCA1 is also impaired in the majority of human glioma models provides clues that :

- this tumour suppressor gene may be involved in gliomagenesis
- upstream partner proteins like ATM may be involved in gliomagenesis, as well
- downstream partner poteines like p16 or p53 may be indirectly used a glioma markers.

In parallel, human normal brain cells appeared impaired in the radiation-induced ATM kinase activity, suggesting an endogenous specificity of brain cells by comparison to other tissues like skin. Since a lower ATM kinase activity and/or expression logically lead to impaired BRCA1 phosphorylation, our data suggest that brain may be more sensitive to tumour formation than other tissue for stress requiring the ATM and BRCA1-dependent pathways (Fig.7).

Our results may also provide interesting elements for anti-glioma strategies. Indeed, since BRCA1 is required for the response to alkylating agents, radio-chemotherapy with platinated agents may be one of the approaches compatible with our findings. Obviously, further molecular and cellular investigations with a larger number of cell lines may consolidate this working hypothesis.

Fig. 7. Schematic recapitulation of our observation. Bold expressions represent the first experimental observations.
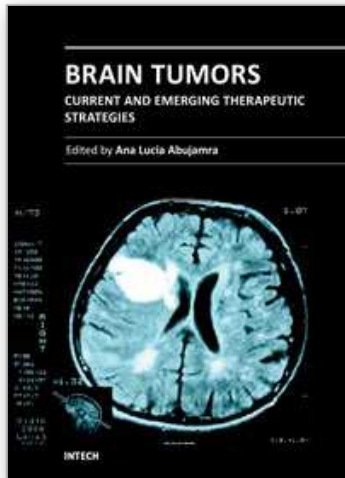
6. Acknowledgments

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Edited by Dr. Ana Lucia Abujamra

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