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THE HUMAN RECQ1 HELICASE IS HIGHLY EXPRESSED IN GLIOBLASTOMA AND PLAYS AN IMPORTANT ROLE IN TUMOR CELL PROLIFERATION [1]

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Abstract — RecQ helicases play an essential role in the maintenance of genome stability. In humans, loss of RecQ helicase function is linked to predisposition to cancer and premature ageing. Distinct molecular functions for the five human RecQ helicases, and RECQ1 in particular, in replication stress and cancer remain to be defined. Within the GLIOMA project we are studying the expression and the role of RECQ1 in replication stress response and repair in glioblastoma and Glioma Stem Cells (GSC). Different papers show that GSCs play an important role in the initiation, aggressiveness and recurrence of glioblastoma. Here, we show that RECQ1 is highly expressed in various types of solid tumours, but only in the case of glioblastoma, the expression of RECQ1 is significantly increased in tumoural tissue relative to the surrounding perilesional. We also show that the depletion of RECQ1 by RNAi results in a significant reduction of cellular proliferation, perturbation of S-phase progression, and spontaneous γ-H2AX foci formation in T98G and U87 glioblastoma cells. Moreover, RECQ1 depleted cells are hypersensitive to hydroxyurea or temozolomide treatment. Collectively, these results indicate that RECQ1 has an important role in the maintenance of genome integrity and might represent a new suitable target for anti cancer therapies aimed to arrest cell proliferation in glioma. In the framework of GLIOMA project we are also working to establish a zebrafish cancer model to characterize the role of RECQ1 in glioblastoma formation and progression *in vivo*.

Index Terms — GLIOMA project, glioblastoma, cancer stem cells, RECQ1 helicase, genome stability

1 BACKGROUND

RecQ helicases are a ubiquitous family of DNA unwinding enzymes involved in the maintenance of chromosome stability. Unwinding of double-stranded DNA is essential for the processes of DNA repair, recombination, transcription, and DNA replication. Five members of the RecQ family have been found in human cells: BLM, RECQ1 (also known as RECQL or RECQL1), RECQ4, RECQ5, and WRN. Three of the five human RecQ genes, BLM, WRN and RECQ4, have been genetically linked to the autosomal recessive diseases Bloom Syndrome (BS), Werner Syndrome (WS) and Rothmund-Thomson Syndrome (RTS), respectively, which display clinical symptoms of premature aging and cancer predisposition. Mutations in the RECQ1 and RECQ5 genes may be responsible for additional cancer predisposition disorders, but this remains to be proven. RECQ1 helicase is the most highly expressed of the human RecQ helicases; however, the biological functions in cellular DNA metabolism are not well known [2].
Glioblastoma (GBM) is the most common and lethal type of primary brain tumours with a median survival of less than 12 months. Despite current therapeutic advances in other solid cancers, the treatment of this malignancy remains essentially palliative. Different findings suggest that a subpopulation of stem cell-like tumour cells, the so called glioma stem cells (GSCs), contribute to tumour initiation, maintenance and recurrence. These stem cell populations have been reported to be radio- and chemo-resistant [3]. Therefore, identification of novel markers for GBM stem cells may lead to better targeting of these cells and thus may impact on the treatment of GBM.
Here we show that RECQ1 is highly expressed in various types of solid tumours, but only in the case of glioblastoma, the expression of RECQ1 is significantly increased in tumoural tissue relative to the surrounding perilesional [1].

2 OBJECTIVES

In the framework of the GLIOMA project our group is studying the expression and the role of RECQ1 in replication stress response and repair in glioma and Glioma Stem Cells (GSCs). Since different evidence prove that this subpopulation of cells contribute to aggressiveness, relapse and treatment resistance of glioblastoma, our main goals within the GLIOMA project are to characterize the expression and function of RECQ1 and identify new biomarkers related to DNA repair pathway. Within this project we also aim to establish a zebrafish cancer model to study the role of helicase in neoplasm formation and in response to DNA-damage inducing agents.

3 APPROACH & METHODS

a) RECQ1 analyses on tissues:
The protein expression profile of RECQ1 was analysed in different type of tumours that were provided by the Pathology Department of the Trieste University and submitted to immunohystochemistry IHC analyses of RECQ1. From this test, RECQ1 resulted to be a candidate biomarker for brain tumors. To explore this hypothesis, 63 additional biopsies of glioblastoma were collected from the archive of the same pathology department and this case study was used to construct a Tissue Microarray (TMA) for the IHC analyses.

b) RECQ1 analyses on cell lines:
To study the function of RECQ1 in replication stress response and repair in glioblastoma, the following cell lines of human origin have been used: T98G and U87 (Glioblastoma cell lines) and the IMR-90 (Normal fibroblast cell line). To study the role of RECQ1 in glioma stem cells we are using the NCH421k stem-like glioma cell lines.

RNA interference technique was used to silencing the RECQ1 gene. The cells depleted for RECQ1 were used for functional study and for the establishment of a zebrafish model. Flow cytometry was used to simultaneously determine the cell-cycle profile (DNA content) by incorporation of propidium iodide and S-phase cell population by incorporation of BrdU.
Clonogenic assay was performed to check cell survival after treatment with hydroxyurea (HU) or Temozolamide (TMZ).

4 RESULTS

a) RECQ1 is significantly increased in the glioblastoma tissues relative to perilesional tissues.
The protein expression profile of RECQ1 was analyzed in different types of tumours. IHC analysis of lesional and perilesional sections of different solid tumour (colon carcinoma, thyroid cancer, lung cancer, and brain glioblastoma tissues) showed that RECQ1 was effectively detected in these samples. However, only in the glioblastoma the expression of RECQ1 was significantly higher in the tumor samples in comparison to the perilesional tissues. In fact, using tissue microarray containing a total of 63 glioblastoma and 19 perilesional tissues the percentage of RECQ1 positive cells were significantly higher in tumoral versus perilesional tissues ($p = 0.001$) (Fig. 1).

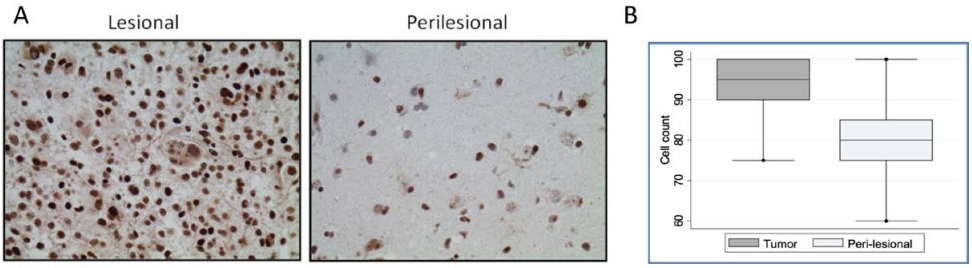

Fig 1. (A) Representative IHC of RECQ1 expression in tissue cores derived from a glioblastoma specimen and from the corresponding peri-lesional surrounding tissue (O.M. 40X). (B) Distribution of cells positive to RECQ1 in 19 brain glioblastomas and paired peri-lesional tissues. The boxes summarize the confidence interval (lower and higher horizontal lines), 25th-50th and 75th percentiles (horizontal lines of the box).

b) RECQ1 is involved in proliferation of glioblastoma cells and are hypersensitive to Temozolamide.
Protein expression analyses of RECQ1 on gliobastoma tissues suggest that the helicase is almost absent in perilesional and/or normal tissues, possibly because of the low degree of proliferation of brain cells. Hence, we believe that RECQ1 could be a potential target for chemotherapy in brain tumours, since its depletion by RNAi or its inhibition by selective compounds could target selectively neoplastic cells. To test the role of RECQ1 in glioblastoma cell growth and proliferation, we compared the colony forming properties of the T98G and U87 glioblastoma cell lines and normal human IMR-90 fibroblasts, after the transient transfection with RECQ1-specific siRNAs, versus cells transfected with a luciferase siRNA as control. The colony forming assays demonstrated a significant reduction in plating efficiencies (expressed as colony forming capacity) of RECQ1 downregulated T98G and U-87 glioblastoma cells (Fig. 2 A). To explore the possibility that RECQ1 might represent a suitable new target for brain tumour treatment, we investigated the sensitivity of glioblastoma cells to temozolamide (TMZ), a commonly used anticancer agent in the treatment of human brain tumours. Cellular survival curves related to increasing TMZ concentrations showed that RECQ1 depleted T98G cell line was hypersensitive to the action of TMZ suggesting a possible role of RECQ1 in DNA repair pathways linked to DNA replication (Fig. 2B).

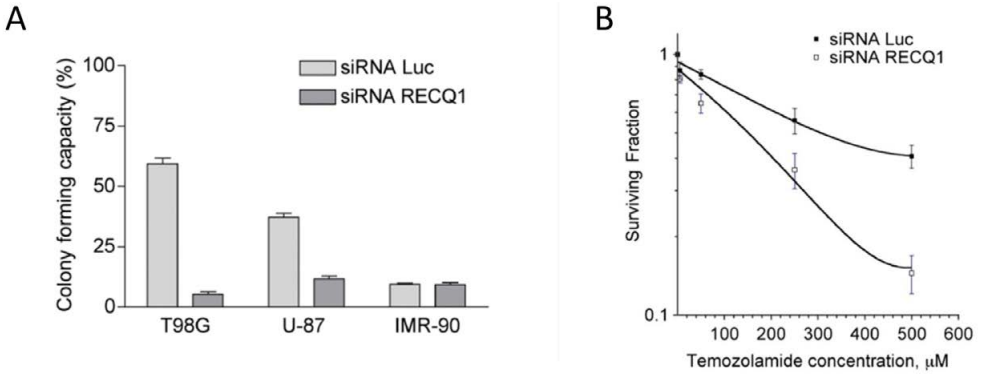

Fig 2. (A) Clonogenic assays performed in RECQ1-depleted T98G, U-87, and IMR-90 cell lines. Bar-graphs show the plating efficiencies expressed as colony forming capacity. (B) The graphs show the cellular surviving fractions measured at different doses of temozolamide in control and RECQ1-depleted T98G. Surviving fraction values are the mean ± SEM from three independent experiments.

c) RECQ1 depletion leads to cell cycle perturbation

Previous studies showed that siRNA-mediated depletion of RECQ1 impaired cellular proliferation in different cell lines. This observation was also supported by FACS analysis of RECQ1-depleted T98G glioblastoma cells that had been bromodeoxyuridine (BrdU)-labelled, indicating that there is more than 50% reduction in S phase fraction. These results confirm that RECQ1 depletion suppresses cell proliferation by interfering with DNA synthesis. Further, the colony forming capacity of RECQ1-depleted cells upon replication stress induction with HU showed that RECQ1 depleted cells were hyper-sensitive to HU treatment, suggesting a possible role of RECQ1 in DNA replication fork processing.

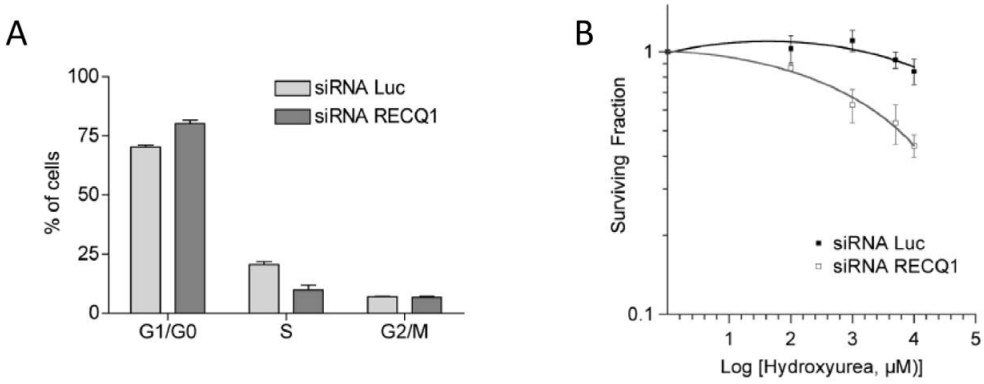

Fig 3. (A) FACS analysis: the bar graph reports the percentage of G0/G1, S-phase/BrdU positive and G2/M cells in cultures that had been transfected with RECQ1 (siRNA RECQ1) or luciferase/control (siRNA Luc) siRNA pools. Results shown are the mean ± SE from three independent experiments. (B) Cellular surviving fractions measured at different doses of HU in control and RECQ1-depleted T98G cells. Surviving fraction values are the mean ± SE from three independent experiments.

d) Preparation of cells with depleted RECQ1 for establishment of zebrafish model
In order to study the role of RECQ1 in glioblastoma formation and in response to DNA-damage inducing agents, we prepared DsRed fluorescent U87 glioma cell line and NCH421k stem-like glioma cell line depleted for RECQ1 to inject in zebrafish embryo. This step will be done in collaboration with NIB (National Institute of Biology, Ljubliana).

5 POTENTIAL NEW PRODUCTS & SERVICES

The final goal of the GLIOMA project is the identification for novel molecular targets and biomarkers for the improvement of diagnosis and the therapy response, as well as for more reliable prognosis of disease progression. Our aim is to identify new cancer stem cell biomarkers related to DNA repair pathway. On the basis of isolated glioblastoma stem cells from tumour tissues of patients, researchers have been able to identify novel biomarkers with the potential to be used for cell-targeting in glioblastoma treatment. Our current results indicate that RECQ1 could be considered as biomarker of GSC, but further validations are necessary. Moreover, the work in progress to establish a zebrafish cancer model will contribute to study the role of RECQ1 in neoplasm formation and in response to DNA-damage inducing agents, and become a valid tool to test novel drugs.

6 CURRENT COLLABORATIONS

Within the GLIOMA project we collaborate with National Institute of Biology, Ljubljana, Slovenia and "Santa Maria della Misericordia" Health-University Institute, Udine, Italy.

7 CONTACT OR COLLABORATIONS NEEDED

Collaboration with an institution that has access to clinical and primary samples is required.

ACKNOWLEDGEMENT

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