Molecular & Cellular Oncology 1:3, e964028; October 1, 2014; © 2014 Taylor & Francis Group, LLC

Functional characterization of ENPP1 reveals a link between cell cycle progression and stem-like phenotype in glioblastoma

Josephine Bageritz[1] and Violaine Goidts[2,*]

[1]The Gurdon Institute and Department of Physiology; Development and Neuroscience; University of Cambridge; Tennis Court Road, Cambridge, UK;
[2]Division of Molecular Genetics; German Cancer Research Center; Heidelberg, Germany

Keywords: cell cycle, differentiation, ENPP1, glioblastoma stem-like cells, high-throughput screen, phosphatase

Abbreviations: GSCs, glioblastoma stem-like cells; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; RNAi, interfering RNA; E2F1, E2F transcription factor 1.

A high-throughput phenotypic screen in glioblastoma stem-like cells (GSCs) identified a novel molecular mechanism in which ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) plays an important role in balancing the pool of nucleotides, thus maintaining GSCs in an undifferentiated proliferative state. This finding highlights the connection between cell cycle length and the stem-like tumor state.

Glioblastoma is the most common primary brain tumor and among the deadliest human cancers. Because of its location, aggressiveness, and diffuse growth pattern, therapy for glioblastoma is tremendously challenging and only a small improvement in survival has been achieved over the past 30 years. Many patients do not efficiently respond to currently available drugs. This is thought to be mainly due to a sub-population of highly resistant tumor cells, the so-called glioblastoma stem-like cells (GSCs), that can continuously self-renew and regenerate the tumor.[1,2]

Since current treatment strategies aim to target the bulk tumor mass they potentially fail to account for the different molecular and clinical properties of this sub-population of GSCs, ultimately causing the therapy to be unsuccessful. This failure might be caused by the different features of bulk tumor cells and GSCs such as variable proliferation rates, differential gene expression including genes that control differentiation, and alterations in programmed cell death. Indeed, major drawbacks of current cancer chemotherapy are the lack of tumor-specific targets and its incapacity to target GSCs. It is therefore critical to discover novel cancer drugs targeting that sub-population of tumor cells in particular.

In the last decade, the advent of high-throughput next-generation sequencing technologies has led to a new era in the study of *de novo* mutations, helping to uncover disease preconditions and mechanisms. However, although very informative, these analyses do not account for post-translational modifications that lead to oncogene activation or tumor suppressor gene inhibition. The use of a high-throughput phenotypic screen is a means to study the activity of tumor-relevant pathways.[3,4]

Using a lentiviral-based RNAi screen, we aimed to identify phosphatases that are implicated in the maintenance of GSCs.[3] This screening approach identified many genes that alter the level of the GSC marker CD133 and are as such potential positive regulators of the GSC phenotype. Among these, we highlighted ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), an ectonucleotidase located at the cell surface whose role is to cleave pyrophosphate and phosphodiester bonds from various substrates. Interestingly, ENPP1 is highly expressed in Grade IV glioblastoma and GSCs compared to normal brain and fetal neural stem cells, suggesting an important role in this aggressive brain tumor. In addition to its effect on CD133, knockdown of *ENPP1* leads to downregulation of other progenitor/stem cell markers such as CD15, LHX2, and MSI1, as well as increased expression of astrocytic differentiation markers. Moreover, comparison of existing stem cell-associated gene sets with the expression profiling of ENPP1-deficient GSCs revealed a global downregulation of stem cell-associated genes. These data support the important role of ENPP1 for the maintenance of the GSC phenotype. As differentiation of GSCs has previously been shown to increase their response to therapeutic agents, knockdown of *ENPP1* increased the apoptotic response of the cells to 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU), a cytotoxic agent used in the treatment of malignant gliomas. Additionally, we have shown that knockdown of *ENPP1* affects

*Correspondence to: Violaine Goidts; E-mail: v.goidts@dkfz.de
Submitted: 07/18/2014; Revised: 08/06/2014; Accepted: 08/12/2014
http://dx.doi.org/10.4161/23723548.2014.964028

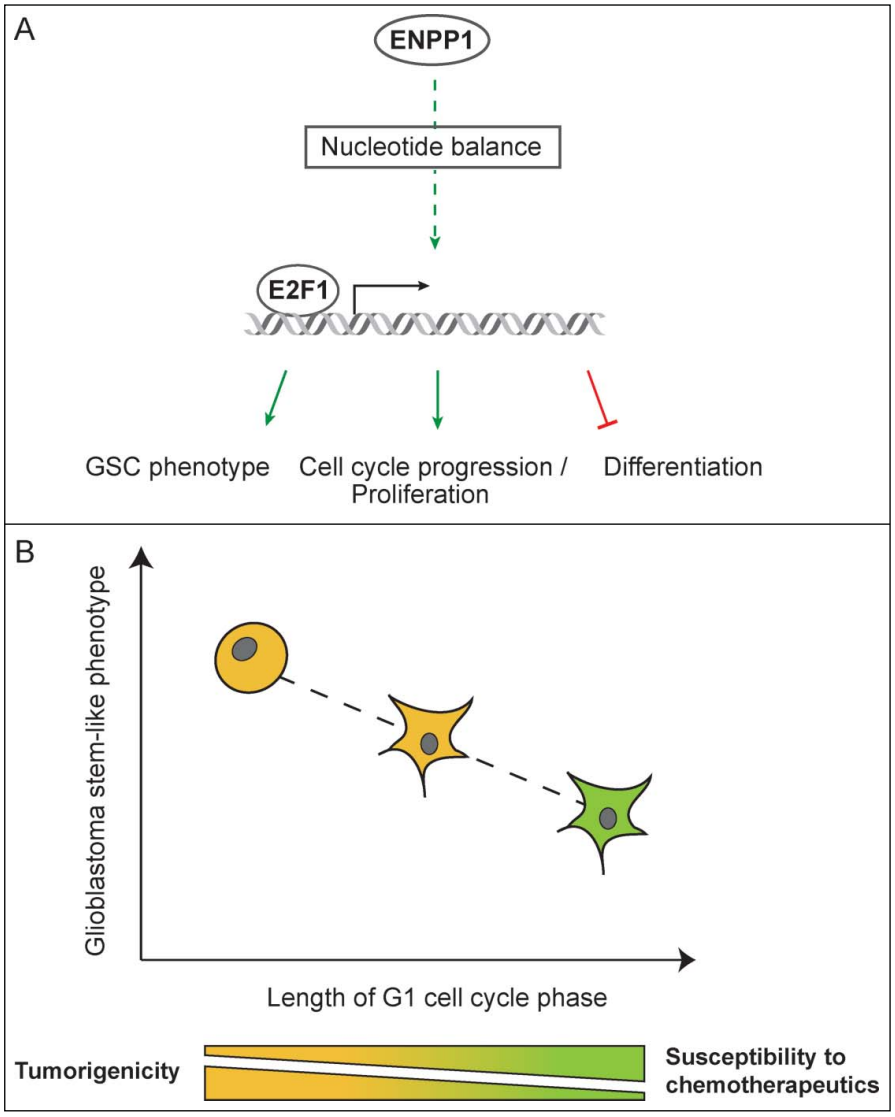

Figure 1. Factors influencing the phenotype of glioblastoma stem-like cells: (**A**) Model of ENPP1 function in glioblastoma stem-like cells. Acting upstream of the E2F transcription factor 1 (E2F1), ENPP1 is important for the maintenance of a proliferative stem-like phenotype in glioblastoma. A possible implication of a balanced nucleotide pool is indicated by the dashed line. (**B**) Proposed model for the interconnection of cell cycle state and stem-like phenotype in glioblastoma and its impact on tumorigenicity and response to chemotherapeutics.

the level of the intracellular nucleotide pool. This deregulation might be attributable to the enzymatic activity of ENPP1 in the extracellular space, resulting in decreased transcriptional function of the cell cycle regulator E2F transcription factor 1 (E2F1) (**Fig. 1A**). Interestingly, *ENPP1* knockdown impaired proliferation and led to the accumulation of cells in G1 phase of the cell cycle. This appears to be a direct consequence of the decreased transcriptional function of E2F1, which is known to positively control S phase entry. Numerous studies have highlighted the correlation between cell cycle progression and cell fate decision in neural stem cells;[5-7] however, our study is the first to suggest the interconnection of a lengthened G1 phase and the induction of differentiation in GSCs. These data are in line with previous findings showing onset of differentiation accompanied by an accumulation of stem-like cells in G1 phase.[8,9] This might have important implications for therapy as the length of the G1 phase of the tumor cells seems to impact tumorigenicity and resistance of these cells to chemotherapeutic agents (**Fig. 1B**).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006; 444:756-60; PMID:17051156; http://dx.doi.org/10.1038/nature05236
2. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2006; 5:67; PMID:17140455; http://dx.doi.org/10.1186/1476-4598-5-67
3. Bageritz J, Puccio L, Piro RM, Hovestadt V, Phillips E, Pankert T, Lohr J, Herold-Mende C, Lichter P, Goidts V. Stem cell characteristics in glioblastoma are maintained by the ecto-nucleotidase E-NPP1. Cell Death Differ 2014; 21:929-40; PMID:24531536; http://dx.doi.org/10.1038/cdd.2014.12
4. Wurdak H, Zhu S, Romero A, Lorger M, Watson J, Chiang CY, Zhang J, Natu VS, Lairson LL, Walker JR, et al. An RNAi screen identifies TRRAP as a regulator of brain tumor-initiating cell differentiation. Cell Stem Cell 2010; 6:37-47; PMID:20085741; http://dx.doi.org/10.1016/j.stem.2009.11.002
5. Lange C, Huttner WB, Calegari F. Cdk4/cyclinD1 overexpression in neural stem cells shortens G1, delays neurogenesis, and promotes the generation and expansion of basal progenitors. Cell Stem Cell 2009; 5:320-31; PMID:19733543; http://dx.doi.org/10.1016/j.stem.2009.05.026
6. Pauklin S, Vallier L. The cell-cycle state of stem cells determines cell fate propensity. Cell 2013; 155:135-47; PMID:24074866; http://dx.doi.org/10.1016/j.cell.2013.08.031
7. Hardwick LJ, Philpott A. Nervous decision-making: to divide or differentiate. Trends Genet 2014; 30:254-61; PMID:24791612; http://dx.doi.org/10.1016/j.tig.2014.04.001
8. Campos B, Wan F, Farhadi M, Ernst A, Zeppernick F, Tagscherer KE, Ahmadi R, Lohr J, Dictus C, Gdynia G, et al. Differentiation therapy exerts antitumor effects on stem-like glioma cells. Clin Cancer Res 2010; 16:2715-28; PMID:20442299; http://dx.doi.org/10.1158/1078-0432.CCR-09-1800
9. Chirasani SR, Sternjak A, Wend P, Momma S, Campos B, Herrmann IM, Graf D, Mitsiadis T, Herold-Mende C, Besser D, et al. Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of stem-like glioblastoma cells. Brain 2010; 133:1961-72; PMID:20513660; http://dx.doi.org/10.1093/brain/awq128