Published in final edited form as:

Leuk Res. 2014 February ; 38(2): 147–148. doi:10.1016/j.leukres.2013.11.011.

G0S2—A new player in leukemia

Kimberly J. Payne* and

Center for Health Disparities and Molecular Medicine, Department of Pathology and Human Anatomy, Loma Linda University School of Medicine, 1st floor Mortensen Hall, 11085 Campus St., Loma Linda, CA 92350, USA

Sinisa Dovat

Department of Pediatrics, Pennsylvania State University College of Medicine, Hershey, PA 17033, USA

Keywords

G0S2; Tumor suppressor; Leukemia; CML; K562

Defining characteristics of cancer cells include deregulated gene expression and a loss of cell cycle control. Thus, to understand the process of oncogenesis and design novel, specific, anticancer therapies, it is essential to: (1) identify genes whose deregulation is associated with uncontrolled cellular proliferation and cell cycle progression; (2) understand the mechanisms by which proteins encoded by these genes regulate cellular proliferation; and (3) determine the mechanisms that regulate expression and/or activity of these genes and alterations in these processes in malignant cells.

G0/G1 switch gene 2 (G0S2) is a small basic protein shown to be involved in lymphocyte activation [1], regulation of lipolysis [2,3], and proliferation of leukemia cells [4], however, its specific cellular functions have remained largely undefined. A pro-apoptotic role for G0S2 has been demonstrated in human primary fibroblasts, where G0S2 interacts with Bcl-2, to promote apoptosis by preventing the formation of Bcl-2/Bax heterodimers [5]. G0S2 has also been shown to inhibit the proliferation of hematopoietic stem cells [4]. A potential mechanism of G0S2 anti-proliferative activity includes the direct interaction of the G0S2 protein with nucleolin in resting cells, leading to cytosolic retention of nucleolin and thus the prevention of nucleolin's pro-proliferative function in the nucleus/nucleolus [4]. These data suggested that G0S2 might have different functions in different tissues, and that it might act as a tumor suppressor.

Additional circumstantial evidence supports a possible role for G0S2 as a tumor suppressor. In head and neck cancers, squamous lung cancer, and cisplatin-resistant cancer cells the G0S2 gene is epigenetically silenced suggesting a role in oncogenesis and resistance to chemotherapy [6,7]. In hematological malignancies, G0S2 is part of the molecular signature

of CD34+ cells in patients with chronic myeloid leukemia (CML) [8,9]. The latter results are particularly intriguing, as they raise the possibility that G0S2 plays a role in leukemia stem cells that give rise to CML, a particular subset of cells that is responsible for chemotherapy resistance and relapse [10].

The above data suggest that the G0S2 gene plays an important role in leukemia (or at least in CML), and that its transcription is tightly regulated in malignant cells. These data also underscore the importance of: (1) identifying whether G0S2 can regulate cellular proliferation; (2) defining the mechanisms by which G0S2 regulates proliferation of leukemia cells; and (3) elucidating the mechanisms that regulate expression of G0S2 in leukemia.

In this issue of Leukemia Research, Yamada et al. provide important insights into these issues. The authors build on their previous work, which suggests a role for G0S2 in regulating proliferation of leukemia stem cells. Here, the authors first establish that transcription of G0S2 is severely reduced in leukemia cells as compared to normal hematopoietic cells. The authors then evaluated DNA methylation as a mechanism for regulating G0S2 expression in leukemia. The proximal promoter sequence's upstream start site, exon 1, and part of the coding sequence of exon 2 of G0S2 were assayed using bisulfite sequencing. Data from these experiments established that G0S2 expression in leukemia cells is regulated through methylation of its promoter.

Next, the authors provide insights into the mechanisms by which G0S2 might regulate cellular proliferation. Using a CML cell line, K562, as a model they show that increased expression of G0S2 following treatment with 5-azacytidine (5-Aza) correlates with the redistribution of nucleolin into a predominantly perinuclear localization, and thus cytosolic retention of nucleolin in K562 cells. The cytosolic retention of nucleolin was strongly associated with cessation of cell growth and proliferation. Evidence that this process is dependent on G0S2 expression, comes from data showing that cells expressing G0S2 shRNA do not show redistribution and cytoplasmic retention of nucleolin when treated with 5-Aza, nor do they show a reduction in cellular proliferation. Indeed, an increase in the percentage of cycling cells was observed when K562 cells expressing G0S2 shRNA were treated with 5-Aza. These results suggest that G0S2 regulates cell cycle progression and cellular proliferation in K562 CML leukemia cells and that the anti-proliferative effect of 5-Aza treatment involves increased expression of G0S2 following demethylation of its promoter.

More direct evidence that G0S2 regulates *in vivo* proliferation of K562 cells was provided by data from a xenograft model. Growth of K562 cells that overexpressed G0S2 was compared to that of control K562 cells, *in vivo*, in a mouse xenograft. Results showed that K562 cells overexpressing G0S2 had much reduced tumor growth and lower levels of mitotic features *in vivo* when compared to control K562 cells. These data provide direct evidence that increased expression of G0S2 results in reduced proliferation of K562 cells *in vivo*, further suggesting a tumor suppressive role for G0S2 in leukemia.

The studies reported by Yamada et al. are significant in several ways. First, this work advances our understanding of the role of G0S2 in leukemia. The presented data strongly support the hypothesis that G0S2 has anti-proliferative activity in leukemia, and that epigenetic regulation (methylation of its promoter) is one of the mechanisms that reduces its expression in leukemia and likely contributes to malignant transformation. Second, these data also suggest that one contributing mechanism to the therapeutic efficacy of 5-Aza in leukemia involves increased expression of G0S2 following demethylation of its promoter. Thus, these data advance our understanding of both oncogenesis and therapeutic mechanisms.

Significant challenges remain in developing a complete picture of the G0S2-mediated tumor suppression suggested by the data described here. Exhaustive analyses of the expression of G0S2 in primary cells of various types of leukemia, as well as confirmation of the results presented by Yamada et al. in other types of leukemia, will provide information on how universal the role of G0S2 is in different types of hematopoietic malignancies. Further studies are also needed to define the specific mechanisms by which G0S2 regulates subcellular localization of nucleolin to aid in developing a better understanding of both of these proteins in leukemia. Finally, additional studies will be needed to determine if the increased expression of G0S2 is the primary mechanism by which 5-Aza exerts its anti-proliferative effect, or whether this is one of many contributory pathways to the therapeutic efficacy of 5-Aza. All of these are important issues that will have to be resolved to get a clear view of the roles of G0S2 and nucleolin in leukemia. The paper by Yamada et al. brings us a step closer to achieving this goal and we can look forward to the continuation of this work.

Acknowledgements

The work has been supported by NIH grant R01 HL095120, a St. Baldrick's Foundation Career Development Award, a Hyundai Hope on Wheels Scholar Grant Award, the Four Diamonds Fund of the Pennsylvania State University College of Medicine, and the John Wawrynovic Leukemia Research Scholar Endowment (to SD). This work was also supported by NIH grant R21 CA162259 and by a Grant to Promote Collaborative and Translational Research from Loma Linda University (KJP) as well as by P20MD006988.

References

1. Russell L, Forsdyke DR. A human putative lymphocyte G0/G1 switch gene containing a CpG-rich island encodes a small basic protein with the potential to be phosphorylated. *DNA Cell Biol.* 1991; 10:581–591. [PubMed: 1930693]
2. Heckmann BL, Zhang X, Xie X, Liu J. The G0/G1 switch gene 2 (G0S2): regulating metabolism and beyond. *Biochim Biophys Acta.* 2013; 1831:276–281. [PubMed: 23032787]
3. Yang X, Lu X, Lombes M, Rha GB, Chi YI, Guerin TM, et al. The G(0)/G(1) switch gene 2 regulates adipose lipolysis through association with adipose triglyceride lipase. *Cell Metab.* 2010; 11:194–205. [PubMed: 20197052]
4. Yamada T, Park CS, Burns A, Nakada D, Lacorazza HD. The cytosolic protein G0S2 maintains quiescence in hematopoietic stem cells. *PloS One.* 2012; 7:e38280. [PubMed: 22693613]
5. Welch C, Santra MK, El-Assaad W, Zhu X, Huber WE, Keys RA, et al. Identification of a protein, G0S2, that lacks Bcl-2 homology domains and interacts with and antagonizes Bcl-2. *Cancer Res.* 2009; 69:6782–6789. [PubMed: 19706769]
6. Kusakabe M, Watanabe K, Emoto N, Aki N, Kage H, Nagase T, et al. Impact of DNA demethylation of the G0S2 gene on the transcription of G0S2 in squamous lung cancer cell lines

with or without nuclear receptor agonists. *Biochem Biophys Res Commun.* 2009; 390:1283–1287. [PubMed: 19878646]

7. Tokumaru Y, Yamashita K, Osada M, Nomoto S, Sun DI, Xiao Y, et al. Inverse correlation between cyclin A1 hypermethylation and p53 mutation in head and neck cancer identified by reversal of epigenetic silencing. *Cancer Res.* 2004; 64:5982–5987. [PubMed: 15342377]
8. Zheng C, Li L, Haak M, Brors B, Frank O, Giehl M, et al. Gene expression profiling of CD34+ cells identifies a molecular signature of chronic myeloid leukemia blast crisis. *Leukemia.* 2006; 20:1028–1034. [PubMed: 16617318]
9. Faber K, Bullinger L, Ragu C, Garding A, Mertens D, Miller C, et al. CDX2-driven leukemogenesis involves KLF4 repression and deregulated PPAR gamma signaling. *J Clin Invest.* 2013; 123:299–314. [PubMed: 23202735]
10. Crews LA, Jamieson CH. Chronic myeloid leukemia stem cell biology. *Curr Hematol Malig Rep.* 2012; 7:125–132. [PubMed: 22467334]