The current results indicate that SSC self-renewal is affected by the genetic background; however, the derivation of GS cells may involve additional factors. We reported that GS cells could be readily established from DBA, B6D2F1 or ICR backgrounds [8]. Although similar cells were reported from other genetic backgrounds including B6 and 129, they grew poorly in culture, and no genetic manipulation of these cells has been reported [13]. In the present study, we were able to establish GS cells from C3H mice, which were sensitive to busulfan-induced damage but responded in a manner distinct from DBA or B6 mice. These GS cells possessed SSC potential, and successful production of transgenic offspring from GS cells of C3H mice indicated that GS cell-based techniques can be applied to a wide range of strains. However, GS cells from the B6 background did not proliferate actively under the same culture conditions, and we have not been able to produce transgenic offspring using plasmid vectors in these mice (our unpublished observation). Thus, there is clearly a need to improve current culture conditions. Although the addition of GFRA1 has been reported to enhance SSC self-renewal [13], it did not have a strong impact in our culture system. The search for additional self-renewal factors must be continued in order to improve the efficacy of the GS cell culture technique.

There are multiple variables that make it difficult to study the genetic differences in self-renewal of SSCs. These include strain differences in 1) sensitivity to busulfan, 2) absolute numbers of endogenous SSCs at any one time, 3) the rate of self-renewal of endogenous SSCs and 4) interactions between SSCs and niche cells or any other somatic cell type that might influence self-renewal of SSCs. The effects of these factors could not be neglected totally in this study. Because SSCs are defined only by their ability to self-renew, they must be assessed by germ cell transplantation, which is the only method available to detect SSCs in a functional manner. We attempted to study the genetic differences of SSCs using this technique. Albeit with several caveats, this study provided a basis for study of the genetic differences of SSCs by functional methods. It also raises several important questions for understanding the kinetics of spermatogenesis. How genetic factors influence SSC behavior *in vivo*, including the regulation of pool size, the life-span and the stress response, remains unknown. For example, studies in the hematopoietic system have revealed fundamental differences between the mechanisms that control the frequency of HSCs and those that regulate mature blood cell numbers [15]. Understanding these regulatory factors is important for elucidating the molecular basis of SSC self-renewal. It is also important to conduct genetic mapping to find genes that influence SSC self-renewal. At present, we only know of a few molecules that promote SSC self-renewal. The analysis of genetic factors may help to identify additional components that modulate SSC self-renewal.

Acknowledgments

We thank Ms Y Ogata for technical assistance. Financial support for this research was provided by the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Japan Society for the Promotion of Science. This work was also supported by the Genome Network Project, Suzuken Memorial Foundation, Kanae Foundation for the Promotion of Medical Science and Japan Science and Technology Agency (CREST).

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