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Transcriptional control of *KIT* gene expression during germ cell development

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ABSTRACT The characterization of the mechanisms that regulate KIT expression in germ cells at different times of their development is important not only in the field of reproduction, but also for a better understanding of the biology of testicular germ cell tumors (TGCTs). Indeed this tyrosine kinase receptor, besides being essential for the survival and proliferation of primordial germ cells (PGCs) and for postnatal spermatogenesis and oogenesis, is also frequently overexpressed or constitutively active due to activating mutations in carcinoma *in situ* of the testis and in seminomas. In this review, I will summarize available data about the transcriptional mechanisms involved in the control of *Kit* expression in the germline. Variable mechanisms, involving different germ cell-specific transcription factors, are operating in the various developmental stages: SOX2 and SOHLH1/2 act as direct positive regulators in PGCs and in postnatal spermatogonia, respectively, whereas PLZF suppresses KIT expression in spermatogonial stem cells. DMRT1, acting through indirect mechanisms, suppresses KIT transcription in fetal gonocytes, while activating it in differentiating spermatogonia.

KEY WORDS: *KIT tyrosine kinase, primordial germ cell, spermatogenesis, testicular germ cell tumor*

Introduction: pleiotropic roles played by the *kit* gene in different stages of gametogenesis

The tyrosine kinase receptor KIT is required for the survival and proliferation of primordial germ cells (PGCs), and is then down-regulated both in fetal oocytes undergoing meiosis and in male gonocytes, which stop to proliferate after germ cell sex determination (Dolci *et al*., 1991; Sette *et al*., 2000, and references therein). After birth, *Kit* plays important roles also during postnatal stages of spermatogenesis. Indeed, in the adult testis KIT is re-expressed in differentiating spermatogonia, but not in spermatogonial stem cells (Manova *et al*., 1990; Sorrentino *et al*., 1991; Yoshinaga *et al*., 1991; Schrans-Stassen *et al*., 1999; Ohta *et al*., 2000). The KIT ligand (Kitl), expressed by Sertoli cells, stimulates proliferation of type A spermatogonia (Rossi *et al*., 1993; Dolci *et al*., 2001), but, together with retinoic acid (RA) it is also important for triggering meiotic entry of type B spermatogonia (Rossi *et al*., 2008; Pellegrini *et al*., 2008). Indeed, a point mutation in the *Kit* gene, which impairs Kitl-mediated activation of phosphatydilinositol 3-kinase, does not cause any significant reduction in PGCs number during embryonic development, nor in spermatogonial stem cell populations, but males are completely sterile for a block in the initial stages of spermatogenesis (Blume-Jensen *et al*., 2000; Kissel *et al*., 2000). RA and other agents inducing spermatogonial differentiation, such as Bone Morphogenetic Protein 4, stimulate *Kit* expression in undifferentiated spermatogonia (Pellegrini *et al*., 2003; Pellegrini *et al*., 2008; Zhou *et al*., 2008). The full-length KIT protein is also expressed in post-natal oocytes, and several reports indicate that it is important for their growth and maturation (Packer *et al*., 1994; Kissel *et al*., 2000; Klinger and De Felici, 2002; Hutt *et al*., 2006).

On the contrary, with the onset of meiosis *Kit* expression in male germ cells ceases at both the RNA and protein levels (Sette *et al*., 2000). Later on, a truncated *Kit* product, TR-KIT (an intracellular protein corresponding to the carboxy-terminal portion of the kinase domain), is specifically expressed in post-meiotic stages of spermatogenesis, and is accumulated in mature spermatozoa both in mice and humans (Sorrentino *et al*., 1991; Rossi *et al*., 1992; Albanesi *et al*., 1996;. Sette *et al*., 1997; Muciaccia *et al*., 2010). In human spermatozoa TR-KIT is located in the equatorial region, the area involved in sperm–egg fusion (Muciaccia *et al*., 2010). Indeed, TR-KIT is a candidate as one of the sperm-borne factors required for egg activation at fertilization (Sette *et al*., 1997, 1998, 2002).

Thus, due to the important role played by the *Kit* gene in various stages of gametogenesis, it is of great interest to dissect and

Abbreviations used in this paper: PGC, primordial germ cell; TGCT, testicular germ cell tumor.

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characterize the mechanisms that regulate its expression in germ cells at different times of their development.

While it is known that TR-KIT expression in spermatids depends on the activation of a cell and developmental stage-specific promoter within intronic regions of both the mouse and the human *Kit* gene (Albanesi *et al*., 1996; Paronetto *et al*., 2004; Muciaccia *et al.,* 2010), much less information has been available for long time about the transcriptional mechanisms which control expression of the full-length receptor in mitotic germ cells and in oocytes.

Since KIT is essential not only for germ cells, but also for haematopoietic stem cells (HSC) and melanoblasts, in which its expression is subjected to tight transcriptional control, the details of the mechanisms controlling *Kit* transcription have been extensively studied in these lineages. In particular, two members of the MYC superfamily of bHLH (Helix-Loop-Helix) transcription factors, TAL1 and MITF, have been shown to regulate *Kit* expression in early haematopoiesis and in melanogenesis, respectively (Krosl *et al*., 1998; Isozaki *et al*., 1994). In the following paragraphs I will summarize more recent findings that have shed some light on the transcriptional mechanisms involved in the control of *Kit* expression in the germline, showing that, as expected, these mechanisms differ in the various developmental stages.

Transcriptional elements within the *kit* gene essential for its expression in the germline

Cairns *et al.,* (2003) originally identified the cis-acting elements in the 5' flanking region and the first intron of the *Kit* gene which are essential for its expression in hematopoietic progenitors and PGCs. They found six DNase I hypersensitive sites (HS1-HS6) within these genomic regions and developed several mouse lines expressing transgenic green fluorescent protein (GFP) under the control of these regulatory elements. A construct driven by the *Kit* promoter and including all 6 HS sites was found to be highly expressed during mouse development in KIT-expressing cells, including PGCs and hematopoietic progenitors. The *Kit* promoter alone (comprising only HS1) was found to be sufficient to drive low-level GFP expression in PGCs, but unable to function in hematopoietic cells. Hematopoietic expression further required the addition of the intronic HS2 fragment. This intronic fragment also greatly potentiated expression of the reporter gene in PGCs. Thus, elements within the first intron act as an enhancer in both lineages. Optimal hematopoietic expression further required more downstream elements within the first intron, which were instead not required for expression in PGCs (Cairns *et al.,* 2003). The mouse transgenic lines obtained with the constructs containing both the *Kit* promoter region and the first intron were found to express GFP also in postnatal germ cells, while those originating from the construct comprising only the promoter region expressed low levels of EGFP only in PGCs, but not in differentiating spermatogonia (Filipponi *et al*., 2007). In the prepuberal testis, the transgenic line expressing EGFP under the control of *Kit* promoter and first intron showed specific expression only in spermatogonia, and faithfully recapitulated endogenous *Kit* expression in these cells (Pellegrini *et al*., 2008). Indeed, EGFP expression (analogously to endogenous KIT protein), was absent at 1 dpp, appeared at 4 dpp in few small spermatogonial foci within the seminiferous tubules, and was spread at 7 dpp in discrete segments of the testicular tubules, showing the characteristic "wavy" expression pattern of mouse spermatogenesis. Thus, 6.9 kb of the *Kit* promoter region and 3.5 kb of the first *Kit* intron contain all the cis-acting elements essential for transcriptional modulation of KIT expression in the germline.

Transcription factors controlling kit expression in the germline

PLZF (promyelocytic leukemia zinc finger, also known as ZFP145, or ZBTB16) is a DNA sequence-specific transcriptional repressor that can exert local and long-range chromatin remodeling activity through the recruitment of DNA histone deacetylases and through the action of several nuclear corepressors (Barna *et al*., 2002). PLZF expression has been identified in a particular subset of postnatal male germ cells, the spermatogonial stem cells, and male *Plzf* knock-out (KO) mice show progressive germ cell depletion due to exhaustion of this cell population, due to the deregulated expression of genes controlling the switch between spermatogonial self-renewal and differentiation (Buaas *et al*., 2004; Costoya *et al*., 2004; Payne and Braun, 2006). Since *Kit* is a hallmark of differentiating spermatogonia, it was easy to hypothesize that it might be a target of PLZF repression. Indeed, it was found that PLZF directly represses the transcription of *Kit* (Filipponi *et al*., 2007). PLZF represses both endogenous *Kit* expression and expression of a reporter gene under the control of *Kit* regulatory elements. A discrete sequence of the *Kit* promoter, required for PLZF-mediated *Kit* transcriptional repression, was demonstrated to be bound by PLZF both *in vivo* and *in vitro*. Moreover, a 3-bp mutation in this PLZF binding site abolishes the responsiveness of the *Kit* promoter to PLZF repression. In agreement with these findings, a significant increase in KIT expression was found in the undifferentiated spermatogonia isolated from *Plzf*KO mice (Filipponi *et al*., 2007). Moreover, RA was shown to trigger spermatogonial differentiation through the direct or indirect downregulation of PLZF (Dann *et al*., 2008), and up-regulation of KIT (Pellegrini *et al*., 2008). Thus, one mechanism by which PLZF maintains the pool of spermatogonial stem cells is actually through a direct repression of *Kit* transcription. Interestingly, PLZF expression was also found to increase progressively in both male and female mouse fetal germ cells coincidently with down-regulation of KIT expression after 13.5 dpc (Barrios *et al*., 2012).

But what are the transcription factors which positively regulate *Kit* expression in the germline? Good candidates were two bHLH transcription factors specifically expressed in germ cells, SOHLH1 (Spermatogenesis and Oogenesis HLH1) and SOHLH2. Both SOHLHs have been involved in the differentiation of spermatogonia and oocytes (Ballow *et al*., 2006a; Ballow *et al*., 2006b; Hao *et al*., 2008; Pangas *et al*., 2006; Toyoda *et al*., 2009; Suzuki *et al*., 2012). In the male, deletion of each transcription factor leads to the disappearance of KIT-expressing spermatogonia in the prepuberal testis, whereas, in the female, both SOHLH1 and SOHLH2 ablations are responsible of oocyte loss in the neonatal ovary. An expression study of SOHLH1 and SOHLH2 during fetal and postnatal development. showed a strong positive correlation between KIT and the two transcription factors in postnatal spermatogonia, but not in PGCs (Barrios *et al*., 2012). SOHLH2 was found enriched mainly in undifferentiated spermatogonia, whereas SOHLH1 expression was maximal in KIT-dependent stages. Expression of SOHLH1, but not SOHLH2, was increased in postnatal mitotic germ cells by treatment with All-trans Retinoic

Acid, a differentiation-inducing analog of RA. Moreover, reporter gene expression driven by sequences contained within the *Kit* promoter and first intron was strongly up-regulated in transfection experiments overexpressing either SOHLH1 or SOHLH2, and co-transfection of both factors showed a cooperative effect (Barrios *et al.*, 2012). *In vivo*, co-immunoprecipitation results evidenced that the two proteins interact and overexpression of both factors increased endogenous KIT expression. *In vitro* experiments further showed that SOHLH1 and SOHLH2 can independently and cooperatively bind a bHLH binding site containing probe designed on discrete sequences of the *Kit* transcriptional regulatory regions. Finally, by chromatin immunoprecipitation (ChIP) analysis, SOHLH1 was found to occupy discrete bHLH binding site containing regions within the *Kit* promoter in spermatogonia chromatin (Barrios *et al.*, 2012; Suzuki *et al.*, 2012). Moreover, the human SOHLH1 gene, but not a deletion mutant associated to non-obstructive azoospermia, was previously shown to transactivate the human *Kit* promoter in transfection experiments (Choi *et al.*, 2010). Interestingly, using conditional gene targeting, it has been shown that loss of the Doublesex-related transcription factor DMRT1 in spermatogonia causes a precocious exit from the spermatogonial program, and that DMRT1 acts in these cells by restricting RA responsiveness, directly repressing transcription of the meiotic inducer STRA8, and activating transcription of SOHLH1, thereby preventing meiosis and promoting spermatogonial development (Matson *et al.*, 2010). A drastic reduction of KIT expression in spermatogonia was evident in testes from *Dmrt1* conditonal KO mice. These data, alltogether, show compelling evidence that SOHLHs directly stimulate *Kit* transcription in postnatal spermatogonia, thus activating the signalling involved in spermatogonia differentiation and spermatogenetic progression both in mice and in humans. Further experiments are required to show whether SOHLHs are required for *Kit* expression also in postnatal oocytes.

What about the mechanisms activating *Kit* transcription in PGCs? As stated above, SOHLHs are not expressed in PGCs at the time that they express *Kit* (Barrios *et al.*, 2012). Thus, a different mechanism must be active in the very early stages of gametogenesis to allow the onset of *Kit* transcription. We recently focused our attention on the possible involvement of the pluripotency-conferring gene *Sox2* (SRY-related HMG box 2). Besides being on of the key transcription factors required for embryonic stem cell pluripotency and formation of induced pluripotent stem cells, SOX2 is expressed in mouse PGCs and postnatal oocytes (Avilion *et al.*, 2003; Western *et al.*, 2005, Campolo *et al.*, 2013), but the role played by this transcription factor during germ cell development and early embryogenesis was unknown. Since *Sox2* ablation causes early embryonic lethality shortly after blastocyst implantation, we generated mice bearing *Sox2*-conditional deletion in germ cells at different stages of their development through the Cre/loxP recombination system. Embryos lacking *Sox2* in PGCs showed a dramatic decrease of germ cell numbers at the time of their specification. At later stages, we found that SOX2 is strictly required for PGC proliferation (Campolo *et al.*, 2013). We have also found that SOX2 stimulates *Kit* expression in PGCs and binds to discrete transcriptional regulatory sequences of this gene, which is known to be essential for PGCs survival and proliferation. Interestingly, ChIP analysis showed that these regulatory regions are present within the *Kit* first intron, which, as stated above, is essential for *Kit* expression in PGCs. On the contrary *Sox2* conditional deletion in meiotic oocytes does not impair postnatal oogenesis and early embryogenesis, indicating that it is not essential for oocyte

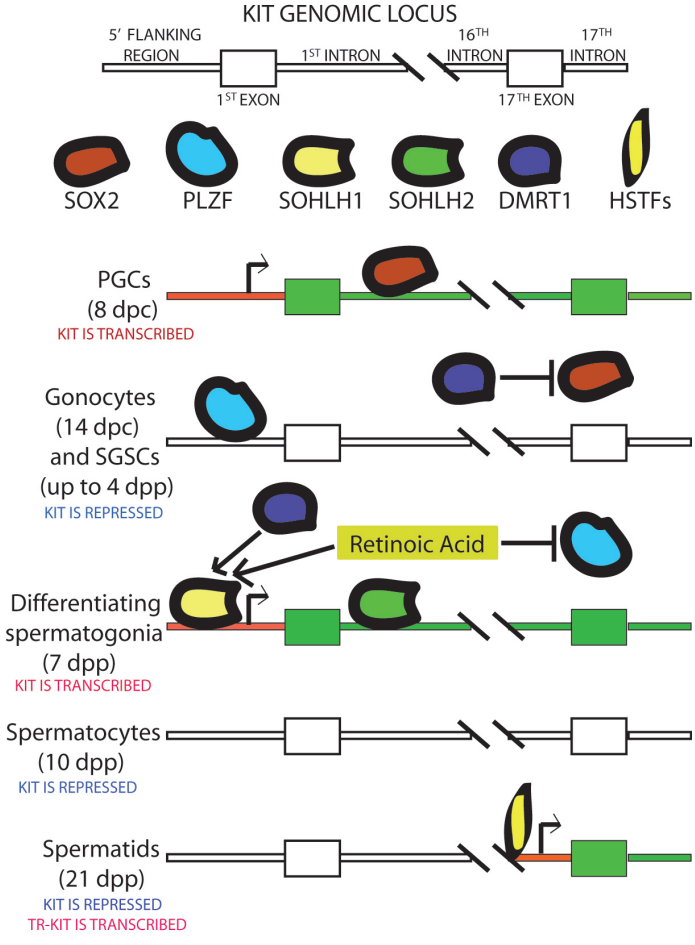

Fig. 1. Schematic representation of the transcriptional regulatory neworks controlling KIT expression in the mouse germline at different developmental stages. *The regulatory regions that are critical for transcription of the two alternative products of the* Kit *gene (the full-length KIT receptor in fetal germ cells and in post-natal spermatogonia, and TR-KIT during spermiogenesis) are depicted, together with the transcription factors known to interact with these genomic regions, which have been discussed in the text. Red colours within the rectangles indicate activation of the alternative promoters at the different developmental stages. Green colours within the rectangles indicate the transcribed areas.within the* Kit *locus. White colours within the rectangles indicate inactive transcription. SOX2 binding in the enhancer area of the first intron activates transcription in early primordial germ cells (PGCs) at 8 days post-coitum (dpc). After sexual determination, in gonocytes and in postnatal spermatogonial stem cells (SGSCs) the transcription factor DMRT1 inhibits SOX2 expression, while binding of the transcriptional repressor PLZF silences the* Kit *promoter up to 4 days post-partum (dpp). At puberty, with the onset of spermatogenesis, the transcription factor SOHLH1, induced by DMRT1 and by Retinoic Acid signalling (which also represses PLZF expression), reactivates full-lenght KIT transcription in differentiating spermatogonia, in collaboration with SOHLH2. Thence, the* Kit *genomic locus is again transcriptionally silent after the mitotic-meiotic switch in spermatocytes. At later stages, activation of a cryptic promoter within the 16t*^{h} Kit *intron by haploid-phase specific transcripion factors (HSTFs) drives TR-KIT expression in sperrmatids and spermatozoa.*

maturation nor for zygotic development, and inferring that SOX2 is not involved in the regaining of *Kit* expression during postnatal oogenesis (Campolo *et al*., 2013).

These results also imply a strict and intricate relationship between stemness and PGCs identity. The transcriptional network regulating *Kit* transcription in the mouse germline at different developmental stages is schematically summarized in Fig. 1.

Future perspectives: possible applications of studies of kit transcriptional regulation for understanding germ cell cancer

The importance of studies on transcriptional conrol of KIT expression in germ cells is not only limited to the field of gametogenesis, but extends also to that of testicular cancer. Indeed, within testicular germ cell tumors (TGCTs), overexpression of KIT is characteristic of seminomas, whereas most nonseminomas do not express KIT (Strohmeyer *et al*., 1995; Bokemeyer *et al*., 1996; Coffey *et al*., 2008). In many cases, KIT overexpression in seminoma might be related to an increase in the copy number of the KIT gene, which occurs in 21% of seminomas but only in 9% of nonseminomas (McIntyre *et al*., 2005). However, alterations of the transcriptional mechanisms that control KIT expression might occur in other cases. Moreover, somatic activating KIT mutations (mainly substitutions in exon 17 at codon 816) are highly frequent in seminomas (as a review, see Coffey *et al*., 2008), implying that pharmacological modulation of mechanisms that regulate germ cell specific KIT expression might have important applications for future therapeutic strategies. This is particularly important, considering that therapies based on direct inhibition of KIT tyrosine kinase activity with imatinib or its derivatives often imply undesired side effects (Gambacorti-Passerini *et al*., 2003), and that frequently KIT-activating mutations are resistant to imatinib therapy (Frost *et al*., 2002). The importance of studies on the transcriptional network controlling germ cell specific KIT expression can be extended also to other germ cell derived tumors. For instance, it has been proposed that testicular teratoma susceptibility in mice might be linked to the lack of *Sox2* repression by DMRT1 in prospermatogonia (Krentz *et al*., 2009). Actually this repression might be one of the key mechanisms underlying down-regulation of KIT expression in male mouse fetal gonocytes after germ cell sexual determination. Eventhough SOX2 has been reported not to be expressed in human PGCs (Perrett *et al*., 2008), these cells have been found to express SOX17 (De Jong *et al*., 2008), which is a srictly related transcription factor showing the same DNA binding properties of SOX2 and interchangeable partner specificity (Jauch *et al*., 2011). Moreover, in conflict with previous reports (Korkola *et al.,* 2006; de Jong *et al.,* 2008; Santagata *et al.,* 2007), SOX2 has been reported to be expressed in human intratubular germ cell carcinoma in situ, the precursor of seminoma (Sonne *et al*., 2010), and in other human TGCTs, such as embryonal carcinoma (Western *et al*., 2005), and the therapeutic potential of SOX2 inhibition by RNA interference in embryonal carcinoma has been recently proposed (Ushida *et al*., 2012).

Clearly, besides transcription, other mechanisms are involved in the regulation of KIT expression in the germline, such as epigenetic events or post-transcriptional control. For instance, it has been reported that all spermatogonia isolated from 8-day-old mice display detectable KIT mRNA, but 30–50% of these lack protein expression (Prabhu *et al*., 2006), presumably implying cell specific translational control mechanisms exerted by microRNA and/or RNA-binding proteins, which are known to play fundamental roles in the regulation of spermatogenesis (Paronetto and Sette, 2010; Messina *et al*., 2012). Very recently miRNA 221, which was previously known to target *Kit* mRNA in hematopoietic stem cells, has been shown to be specifically expressed in spermatogonial stem cells (Smorag *et al*., 2012), and evidence has been given that both KIT mRNA and KIT protein abundance are influenced by miRNA 221 and miRNA 222 function in spermatogonia (Yang *et al.,* 2013). These results imply that, together with repression of *Kit* transcription exerted by PLZF, microRNAs actually contribute to post-transcriptional silencing of *Kit* expression in the spermatogonial stem cell pool.

References

ALBANESI, C., GEREMIA, R., GIORGIO, M., DOLCI, S., SETTE, C. and ROSSI, P. (1996). A cell- and developmental stage-specific promoter drives the expression of a truncated c-kit protein during mouse spermatid elongation. *Development* 122: 1291-1302.

AVILION, A. A., NICOLIS, S. K., PEVNY, L. H., PEREZ, L., VIVIAN, N. and LOVELL-BADGE, R. (2003). Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* 17: 126-140.

BALLOW, D., MEISTRICH, M. L., MATZUK, M. and RAJKOVIC, A. (2006a). Sohlh1 is essential for spermatogonial differentiation. *Dev. Biol.* 294: 161-167.

BALLOW, D. J., XIN, Y., CHOI, Y., PANGAS, S. A. and RAJKOVIC, A. (2006b). Sohlh2 is a germ cell-specific bHLH transcription factor. *Gene Expr. Patterns* 6: 1014-1018.

BARNA, M., MERGHOUB, T., COSTOYA, J. A., RUGGERO, D., BRANFORD, M., BERGIA, A., SAMORI, B. and PANDOLFI, P. P. (2002). Plzf mediates transcriptional repression of HoxD gene expression through chromatin remodeling. *Dev. Cell* 3: 499–510.

BARRIOS, F., FILIPPONI, D., CAMPOLO, F., GORI, M., BRAMUCCI, F., PELLEGRINI, M., OTTOLENGHI, S., ROSSI, P., JANNINI, E.A. and DOLCI, S. (2012). SOHLH1 and SOHLH2 control Kit expression during postnatal male germ cell development. *J. Cell Sci.* 125: 1455-1464.

BLUME-JENSEN, P., JIANG, G., HYMAN, R., LEE, K.F., O'GORMAN, S. and HUNTER, T. (2000). Kit/stem cell factor receptor-induced activation of phosphatidylinositol 3'-kinase is essential for male fertility. *Nat. Genet.* 24: 157–162.

BOKEMEYER, C., KUCZYK, M.A., DUNN, T., SERTH, J., HARTMANN, K., JONASSON, J., PIETSCH, T., JONAS, U. and SCHMOLL, H.J. (1996). Expression of stem-cell factor and its receptor c-kit protein in normal testicular tissue and malignant germ-cell tumours. *J. Cancer Res. Clin. Oncol.* 122: 301-306.

BUAAS, F. W., KIRSH, A. L., SHARMA, M., MCLEAN, D. J., MORRIS, J. L., GRISWOLD, M. D., DE ROOIJ, D. G. and BRAUN, B. E. (2004). Plzf is required in adult male germ cells for stem cell self-renewal. *Nat. Genet.* 36: 647–652.

CAIRNS, L. A., MORONI, E., LEVANTINI, E., GIORGETTI, A., KLINGER, F. G., RONZONI, S., TATANGELO, L., TIVERON, C., DE FELICI, M., DOLCI, S., MAGLI, M.C., GIGLIONI, B. and OTTOLENGHI, S. (2003). Kit regulatory elements required for expression in developing hematopoietic and germ cell lineages. *Blood* 102: 3954-3962.

CAMPOLO, F., GORI, M., FAVARO, R., NICOLIS, S., PELLEGRINI, M., BOTTI, F., ROSSI, P., JANNINI, E.A. and DOLCI, S. (2013). Essential role of Sox2 for the establishment and maintenance of the germ cell line. Stem Cells DOI: 10.1002/stem.1392

CHOI, Y., JEON, S., CHOI, M., LEE, M.H., PARK, M., LEE, D.R., JUN, K.Y., KWON, Y., LEE, O.H., SONG, S.H., KIM, J.Y., LEE, K.A., YOON, T.K., RAJKOVIC, A. and SHIM, S.H. (2010). Mutations in SOHLH1 gene associate with nonobstructive azoospermia. *Hum. Mutat.* 31: 788-793.

COFFEY, J., LINGER, R., PUGH, J., DUDAKIA, D., SOKAL, M., EASTON, D.F., TIMOTHY BISHOP, D., STRATTON, M., HUDDART, R. and RAPLEY, E.A. (2008). Somatic KIT mutations occur predominantly in seminoma germ cell tumors and are not predictive of bilateral disease: report of 220 tumors and review of literature. *Genes Chromosomes Cancer* 47: 34-42.

COSTOYA, J. A., HOBBS, R. M., BARNA, M., CATTORETTI, G., MANOVA, K., SUKHWAN, M., ORWIG, K. E., WOLGEMUTH, D. J. and PANDOLFI, P. P. (2004). Essential role of Plzf in maintenance of spermatogonial stem cells. *Nat. Genet.* 36: 653–659.

DANN, C.T., ALVARADO, A.L., MOLYNEUX, L.A., DENARD, B.S., GARBERS, L. and PORTEUS, M.H. (2008). Spermatogonial stem cell self-renewal requires OCT4, a factor downregulated during retinoic acid-induced differentiation. *Stem Cells* 26: 2928-2937.

DE JONG, J,. STOOP, H., GILLIS, A.J., VAN GURP, R.J., VAN DE GEIJN, G.J., BOER, M., HERSMUS, R., SAUNDERS, P.T., ANDERSON, R.A., OOSTERHUIS, J.W. and LOOIJENGA, L.H. (2008). Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. *J. Pathol.* 215: 21-30.

DOLCI, S., WILLIAMS, D.E., ERNST, M.K., RESNICK, J.L., BRANNAN, C.I., LOCK, L.F., LYMAN, S.D., BOSWELL, H.S. and DONOVAN, P.J. (1991). Requirement for mast cell growth factor for primordial germ cell survival in culture. *Nature* 352: 809- 811.

DOLCI, S., PELLEGRINI, M., DI AGOSTINO, S., GEREMIA, R. and ROSSI, P. (2001). Signaling through extracellular signal-regulated kinase is required for spermatogonial proliferative response to stem cell factor. *J. Biol. Chem.* 276: 40225–40233.

FILIPPONI, D., HOBBS, R. M., OTTOLENGHI, S., ROSSI, P., JANNINI, E. A., PANDOLFI, P. P. and DOLCI, S. (2007). Repression of kit expression by Plzf in germ cells. *Mol. Cell. Biol.* 27: 6770-6781.

FROST, M.J., FERRAO, P.T., HUGHES, T.P. and ASHMAN, L.K. (2002). Juxtamembrane mutant V560GKit is more sensitive to Imatinib (STI571) compared with wild-type c-kit whereas the kinase domain mutant D816VKit is resistant. *Mol. Cancer Ther.* 1: 1115-1124.

GAMBACORTI-PASSERINI, C., TORNAGHI, L., CAVAGNINI, F., ROSSI, P., PECORI-GIRALDI, F., MARIANI, L., CAMBIAGHI, N., POGLIANI, E., CORNEO, G. and GNESSI, L. (2003). Gynaecomastia in men with chronic myeloid leukaemia after imatinib. *Lancet* 361:1954-1956.

HAO, J., YAMAMOTO, M., RICHARDSON, T. E., CHAPMAN, K. M., DENARD, B. S., HAMMER, R. E., ZHAO, G. Q. and HAMRA, F. K. (2008). Sohlh2 knockout mice are male-sterile because of degeneration of differentiating type A spermatogonia. *Stem Cells* 26: 1587-1597.

HUTT, K.J., MCLAUGHLIN, E.A. and HOLLAND, M.K. (2006). KIT/KIT ligand in mammalian oogenesis and folliculogenesis: roles in rabbit and murine ovarian follicle activation and oocyte growth. *Biol. Reprod.* 75: 421-433.

ISOZAKI, K., TSUJIMURA, T., NOMURA, S., MORII, E., KOSHIMIZU, U., NISHIMUNE, Y. and KITAMURA, Y. (1994). Cell type-specific deficiency of c-kit gene expression in mutant mice of mi/mi genotype. *Am. J. Pathol.* 145: 827-836.

JAUCH, R., AKSOY, I., HUTCHINS, A.P., NG, C.K., TIAN, X.F., CHEN, J., PALASINGAM, P., ROBSON, P., STANTON, L.W. and KOLATKAR, P.R. (2011).Conversion of Sox17 into a pluripotency reprogramming factor by reengineering its association with Oct4 on DNA. *Stem Cells* 29: 940-951.

KISSEL, H., TIMOKHINA, I., HARDY, M.P., ROTHSCHILD, G., TAJIMA, Y., SOARES, V., ANGELES, M., WHITLOW, S.R., MANOVA, K. and BESMER, P. (2000). Point mutation in kit receptor tyrosine kinase reveals essential roles for kit signaling in spermatogenesis and oogenesis without affecting other kit responses. *EMBO J.*19: 1312–1326.

KLINGER, F.G. and DE FELICI, M. (2002). *In vitro* development of growing oocytes from fetal mouse oocytes: stage-specific regulation by stem cell factor and granulosa cells. *Dev. Biol.* 244: 85-95.

KORKOLA, J.E., HOULDSWORTH, J., CHADALAVADA, R.S., OLSHEN, A.B., DOBRZYNSKI, D., REUTER, V.E., BOSL, G.J. and CHAGANTI, R.S. (2006). Down-regulation of stem cell genes, including those in a 200-kb gene cluster at 12p13.31, is associated with *in vivo* differentiation of human male germ cell tumors. *Cancer Res.* 66: 820-827.

KRENTZ, A.D., MURPHY, M.W., KIM, S., COOK, M.S., CAPEL, B., ZHU, R., MATIN, A., SARVER, A.L., PARKER, K.L., GRISWOLD, M.D., LOOIJENGA, L.H., BARDWELL, V.J. and ZARKOWER, D. (2009). The DM domain protein DMRT1 is a dose-sensitive regulator of fetal germ cell proliferation and pluripotency. *Proc. Natl. Acad. Sci. USA.* 106: 22323-22328.

KROSL, G., HE, G., LEFRANCOIS, M., CHARRON, F., ROMEO, P. H., JOLICOEUR, P., KIRSCH, I. R., NEMER, M. and HOANG, T. (1998). Transcription factor SCL is required for c-kit expression and c-Kit function in hemopoietic cells. *J. Exp. Med.* 188: 439-450.

MANOVA, K., NOCKA, K., BESMER, P. and BACHVAROVA, R.F. (1990). Gonadal expression of c-kit encoded at the W locus of the mouse. *Development* 110: 1057- 1069.

MATSON, C.K., MURPHY, M.W., GRISWOLD, M.D., YOSHIDA, S., BARDWELL, V.J. and ZARKOWER, D. (2010). The mammalian doublesex homolog DMRT1 is a transcriptional gatekeeper that controls the mitosis versus meiosis decision in male germ cells. *Dev. Cell* 19: 612-624.

MCINTYRE, A., SUMMERSGILL, B., GRYGALEWICZ, B., GILLIS, A.J., STOOP, J., VAN GURP, R.J., DENNIS,N., FISHER, C., HUDDART, R., COOPER, C., CLARK, J., OOSTERHUIS, J.W., LOOIJENGA, L.H. and SHIPLEY, J. (2005). Amplification and overexpression of the KIT gene is associated with progression in the seminoma subtype of testicular germ cell tumors of adolescents and adults. *Cancer Res.* 65: 8085-8089.

MESSINA, V., MEIKAR, O., PARONETTO, M.P., CALABRETTA, S., GEREMIA, R., KOTAJA, N. and SETTE, C. (2012). The RNA binding protein SAM68 transiently localizes in the chromatoid body of male germ cells and influences expression of select microRNAs. *PLoS One* 7: e39729.

MUCIACCIA, B., SETTE, C., PARONETTO, M.P., BARCHI, M., PENSINI, S., D'AGOSTINO, A., GANDINI, L., GEREMIA, R., STEFANINI, M. and ROSSI, P. (2010). Expression of a truncated form of KIT tyrosine kinase in human spermatozoa correlates with sperm DNA integrity. *Hum. Reprod.* 25: 2188-2202.

OHTA, H., YOMOGIDA, K., DOHMAE, K. and NISHIMUNE, Y. (2000). Regulation of proliferation and differentiation in spermatogonial stem cells: the role of c-kit and its ligand SCF. *Development* 127: 2125–2131.

PACKER, A.I, HSU, Y.C., BESMER, P. and BACHVAROVA, R.F. (1994). The ligand of the c-kit receptor promotes oocyte growth. *Dev. Biol.* 161: 194-205.

PANGAS, S. A., CHOI, Y., BALLOW, D. J., ZHAO, Y., WESTPHAL, H., MATZUK, M. M. and RAJKOVIC, A. (2006). Oogenesis requires germ cell-specific transcriptional regulators Sohlh1 and Lhx8. *Proc. Natl. Acad. Sci. USA* 103: 8090-8095.

PARONETTO, M.P., FARINI, D., SAMMARCO, I., MATURO, G., VESPASIANI, G., GEREMIA, R., ROSSI, P. and SETTE, C. (2004). Expression of a truncated form of the c-kit tyrosine kinase receptor and activation of Src kinase in human prostatic cancer. *Am. J. Pathol.* 164: 1243-1251.

PARONETTO, M.P. and SETTE, C. (2010). Role of RNA-binding proteins in mammalian spermatogenesis. *Int. J. Androl.* 33: 2-12.

PAYNE, C. AND BRAUN R. E. (2006). Histone lysine trimethylation exhibits a distinct perinuclear distribution in Plzf-expressing spermatogonia. *Dev. Biol.* 293: 461–472.

PELLEGRINI, M., GRIMALDI, P., ROSSI, P., GEREMIA, R. and DOLCI, S. (2003). Developmental expression of BMP4/ALK3/SMAD5 signaling pathway in the mouse testis: a potential role of BMP4 in spermatogonia differentiation. *J. Cell Sci.* 116: 3363-3372.

PELLEGRINI, M., FILIPPONI, D., GORI, M., BARRIOS, F., LOLICATO, F., GRIMALDI, P., ROSSI, P., JANNINI, E.A., GEREMIA, R. and DOLCI, S. (2008) ATRA and KL promote differentiation toward the meiotic program of male germ cells. *Cell Cycle* 7: 3878-3888.

PERRETT, R.M., TURNPENNY, L., ECKERT, J.J., O'SHEA, M., SONNE, S.B., CAMERON, I.T., WILSON, D.I., RAJPERT-DE MEYTS, E. and HANLEY, N.A. (2008). The early human germ cell lineage does not express SOX2 during *in vivo* development or upon *in vitro* culture. *Biol. Reprod.* 78: 852-858.

PRABHU, S.M., MEISTRICH, M.L., MCLAUGHLIN, E.A., ROMAN, S.D., WARNE, S., MENDIS, S., ITMAN, C. and LOVELAND, K.L. (2006). Expression of c-Kit receptor mRNA and protein in the developing, adult and irradiated rodent testis. *Reproduction* 131: 489-499.

ROSSI, P., MARZIALI, G., ALBANESI, C., CHARLESWORTH, A., GEREMIA, R. and SORRENTINO, V. (1992). A novel c-kit transcript, potentially encoding a truncated receptor, originates within a kit gene intron in mouse spermatids. *Dev. Biol.* 152: 203-207.

ROSSI, P., DOLCI, S., ALBANESI, C., GRIMALDI, P., RICCA, R. and GEREMIA, R. (1993). FSH induction of Steel Factor (SLF) mRNA in mouse Sertoli cells and stimulation of DNA synthesis in spermatogonia by soluble SLF. *Dev. Biol.* 155: 68-74.

ROSSI, P., LOLICATO, F., GRIMALDI, P., DOLCI, S., DI SAURO, A., FILIPPONI, D. and GEREMIA, R. (2008). Transcriptome analysis of differentiating spermatogonia stimulated with kit ligand. *Gene Expr. Patterns* 8: 58-70.

SANTAGATA, S., LIGON, K.L. and HORNICK, J.L. (2007). Embryonic stem cell transcription factor signatures in the diagnosis of primary and metastatic germ cell tumors. *Am. J. Surg. Pathol.* 31: 836-845.

SCHRANS-STASSEN, B.H., VAN DE KANT, H.J., DE ROOIJ, D.G.and VAN PELT,

A.M. (1999). Differential expression of c-kit in mouse undifferentiated and differentiating type A spermatogonia. *Endocrinology* 140: 5894–5900.

SETTE, C., BEVILACQUA, A., BIANCHINI, A., MANGIA, F., GEREMIA, R. and ROSSI, P. (1997). Parthenogenetic activation of mouse eggs by microinjection of a truncated c-kit tyrosine kinase present in spermatozoa. *Development* 124: 2267-2274.

SETTE, C., BEVILACQUA, A., GEREMIA, R. and ROSSI, P. (1998). Involvement of phospholipase C γ1 in mouse egg activation induced by a truncated form of the c-kit tyrosine kinase present in spermatozoa. *J. Cell Biol.* 142: 1063-1074.

SETTE, C., DOLCI, S., GEREMIA, R. and ROSSI, P. (2000). The role of stem cell factor and of alternative c-kit gene products in the establishment, maintenance and function of germ cells. *Int. J. Dev. Biol.* 44: 599–608.

SETTE, C., PARONETTO, M.P., BARCH,I M., BEVILACQUA, A., GEREMIA, R. and ROSSI, P. (2002). Tr-kit-induced resumption of the cell cycle in mouse eggs requires activation of a Src-like kinase. *EMBO J.* 21: 5386-5395.

SMORAG, L., ZHENG, Y., NOLTE, J., ZECHNER, U., ENGEL, W. and PANTAKANI, D.V. (2012). MicroRNA signature in various cell types of mouse spermatogenesis: Evidence for stage-specifically expressed miRNA-221, -203 and -34b-5p mediated spermatogenesis regulation. *Biol. Cell* 104: 677-692.

SONNE, S.B., PERRETT, R.M., NIELSEN, J.E., BAXTER, M.A., KRISTENSEN, D.M., LEFFERS, H., HANLEY, N.A. and RAJPERT-DE-MEYTS, E. (2010). Analysis of SOX2 expression in developing human testis and germ cell neoplasia. *Int. J. Dev. Biol.* 54: 755-760.

SORRENTINO, V., GIORGI, M., GEREMIA, R., BESMER, P. and ROSSI, P. (1991). Expression of the c-kit proto-oncogene in the murine male germ cells. *Oncogene* 6: 149-151.

STROHMEYER, T., REESE, D., PRESS, M., ACKERMANN, R., HARTMANN, M. and SLAMON, D. (1995). Expression of the c-kit proto-oncogene and its ligand stem cell factor (SCF) in normal and malignant human testicular tissue. *J. Urol.* 153: 511-515.

SUZUKI, H., AHN, H.W., CHU,T., BOWDEN, W., GASSEI, K., ORWIG, K, and RAJKOVIC, A. (2012). SOHLH1 and SOHLH2 coordinate spermatogonial differentiation. *Dev. Biol.* 361: 301-312.

TOYODA, S., MIYAZAKI, T., MIYAZAKI, S., YOSHIMURA, T., YAMAMOTO, M., TASHIRO, F., YAMATO, E. and MIYAZAKI, J. (2009). Sohlh2 affects differentiation of KIT positive oocytes and spermatogonia. *Dev. Biol.* 325: 238-248.

USHIDA, H., CHANO, T., MINAMI, K., KITA, H., KAWAKAMI, T., OKABE, H., OKADA, Y. and OKAMOTO, K. (2012). Therapeutic potential of SOX2 inhibition for embryonal carcinoma. *J. Urol.* 187: 1876-1881.

WESTERN, P., MALDONADO-SALDIVIA, J., VAN DEN BERGEN, J., HAJKOVA, P., SAITOU, M., BARTON, S. and SURANI, M. A. (2005). Analysis of Esg1 expression in pluripotent cells and the germline reveals similarities with Oct4 and Sox2 and differences between human pluripotent cell lines. *Stem Cells* 23: 1436-1442.

YANG, Q.-E., RACICOT, K.E., KAUCHER, A.V., OATLEY, M.J. AND OATLEY, J.M. (2013). MicroRNAs 221 and 222 regulate the undifferentiated state in mammalian male germ cells. *Development* 140: 280-290.

YOSHINAGA, K., NISHIKAWA, S., OGAWA, M., HAYASHI, S., KUNISADA, T., FUJIMOTO, T. and NISHIKAWA, S.-I. (1991). Role of c-kit in mouse spermatogenesis: identification of spermatogonia as a specific site of c-kit expression and function. *Development* 113: 689-699.

ZHOU, Q., LI, Y., NIE, R., FRIEL, P., MITCHELL, D., EVANOFF, R.M., POUCHNIK, D., BANASIK, B., MCCARREY, J.R., SMALL, C. and GRISWOLD, M.D. (2008). Expression of stimulated by retinoic acid gene 8 (Stra8) and maturation of murine gonocytes and spermatogonia induced by retinoic acid in vitro. *Biol. Reprod.* 78: 537-45.

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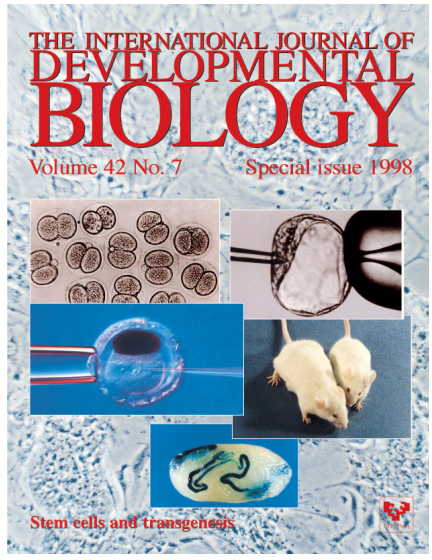

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