



Origin of pluripotent germ cell tumours: The role of microenvironment during embryonic development

David Møbjerg Kristensen, Si Brask Sonne, Anne Marie Ottesen, Rebecca M. Perrett, John E. Nielsen, Kristian Almstrup, Niels E. Skakkebaek, Henrik Leffers, Ewa Rajpert-De Meyts

► To cite this version:

David Møbjerg Kristensen, Si Brask Sonne, Anne Marie Ottesen, Rebecca M. Perrett, John E. Nielsen, et al.. Origin of pluripotent germ cell tumours: The role of microenvironment during embryonic development. Molecular and Cellular Endocrinology, 2008, 288 (1-2), pp.111. <10.1016/j.mce.2008.02.018>. <hal-00532006>

HAL Id: hal-00532006

<https://hal.science/hal-00532006v1>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Accepted Manuscript

Title: Origin of pluripotent germ cell tumours: The role of microenvironment during embryonic development

Authors: David Møbjerg Kristensen, Si Brask Sonne, Anne Marie Ottesen, Rebecca M. Perrett, John E. Nielsen, Kristian Almstrup, Niels E. Skakkebaek, Henrik Leffers, Ewa Rajpert-De Meyts



PII: S0303-7207(08)00106-8
DOI: doi:10.1016/j.mce.2008.02.018
Reference: MCE 6831

To appear in: *Molecular and Cellular Endocrinology*

Received date: 8-11-2007
Revised date: 7-2-2008
Accepted date: 25-2-2008

Please cite this article as: Kristensen, D.M., Sonne, S.B., Ottesen, A.M., Perrett, R.M., Nielsen, J.E., Almstrup, K., Skakkebaek, N.E., Leffers, H., Meyts, E.R.-D., Origin of pluripotent germ cell tumours: The role of microenvironment during embryonic development, *Molecular and Cellular Endocrinology* (2007), doi:10.1016/j.mce.2008.02.018

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**Origin of pluripotent germ cell tumours:
The role of microenvironment during embryonic development**

*David Møbjerg Kristensen¹, Si Brask Sonne¹, Anne Marie Ottesen¹, Rebecca M. Perrett², John E. Nielsen¹, Kristian Almstrup¹, Niels E. Skakkebaek¹, Henrik Leffers¹ & Ewa Rajpert-De Meyts¹

¹University Department of Growth and Reproduction, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

²Centre for Human Development, Stem Cells & Regeneration/Human Genetics Division, University of Southampton, Southampton, SO16 6YD, UK.

*) Corresponding author

David Møbjerg Kristensen, e-mail: moebjerg@imbg.ku.dk

Keywords: Germ cell differentiation, pluripotency, stem cells, cancer stem cells, germ cell neoplasm, carcinoma in situ testis

Abbreviations: Carcinoma *in situ*, CIS; testicular germ cell tumour, TGCT; embryonal carcinoma, EC; primordial germ, PG; testicular dysgenesis syndrome, TDS; embryonic stem, ES.

Abstract

Carcinoma *in situ* (CIS) testis, known also as intratubular germ cell neoplasia, is the cancer stem cell from which testicular germ cell derived tumours (TGCTs) of the testis arise. TGCTs can proliferate into morphologically homogeneous seminomas or can differentiate into virtually any type of tissue and form teratomas (non-seminomas). CIS cells display a close phenotypic similarity to fetal germ cells (primordial germ cells or gonocytes) suggesting an origin due to a developmental delay or arrest of differentiation of early germ cells. The pluripotency of these neoplasms has recently been explained by a close resemblance of their expression profile to that of embryonic inner cell mass cells studied in culture as embryonic stem cells, with high expression of transcription factors associated with pluripotency, such as NANOG and OCT3/4, as well as proteins found in several tissue specific stem cells, such as TFAP2C (AP-2 γ) or KIT. CIS and seminomas highly express a number of pre-meiotic germ cell specific genes, which are down-regulated during development to non-seminomas, while the expression of other embryonic markers, such as SOX2, is up-regulated. The mechanistic pathways and causative factors remain to be elucidated of both the initial transformation of fetal germ cells into CIS cells and the progression of CIS cells into an invasive tumour in the young adult. However, evidence supported by epidemiological studies indicate that disturbances in the hormonal microenvironment of the differentiating gonads may results in both the neoplasia and a host of other problems later in life, such as genital malformations, decreased spermatogenesis, and signs of hypogonadism.

1. Introduction

More than 35 years ago the first paper describing atypical spermatogonia in testicular biopsies from two patients was published (Skakkebaek, 1972a). Both patients later developed overt testicular tumours indicating that the abnormal cells were precursor cells of the malignant tumours (Skakkebaek, 1972b). This original study is to our knowledge one of the first descriptions of a true tumour/cancer stem cell (Nielsen et al., 1974). Today, CIS (also known as intratubular germ cell neoplasia or testicular intraepithelial neoplasia) is generally accepted as the common pre-invasive precursor cell that gives rise to testicular germ cell tumours (TGCTs) of the adolescents and young adults (Ulbright et al., 1999; Oosterhuis and Looijenga, 2005). There are two other types of TGCTs, which develop without a CIS stage: spermatocytic seminoma of elderly men and infantile germ cell tumours. Both of these tumour types are rare and have remained at a steady low incidence level (Visfeldt et al., 1994). The opposite is true for TGCTs, originating from the CIS precursor stage, which have increased in incidence over the last several decades and now constitute the most prevalent malignant cancer among young male adults in Northern and Western Europe, with a life time frequency in Denmark of approximately 1% (Richiardi et al., 2004).

The TGCTs originating from CIS are traditionally subdivided into two histologically distinct groups, seminomas (also called classical seminoma to distinguish from spermatocytic seminoma) and non-seminomatous germ cell tumours, each constituting approximately 50%. Whereas the seminoma is a generally uniform tumour and resembles early fetal germ cells and CIS cells, the more malignant non-seminomas are very heterogeneous and encompass embryonal carcinoma (EC), choriocarcinoma, teratomas, yolk sac tumour, and can also contain elements of seminoma (Ulbright et al., 1999). EC cells of the non-seminomas have the ability to differentiate into a wide range of cell types, and show a phenotypic resemblance to the inner cell mass cells and epiblast from the early embryo. Hence, EC cells represent a malignant caricature of the human embryonic stem (ES) cells that are derived in culture from the inner cell mass (Andrews, 1998; Solter, 2006). However, many EC cells show limited developmental potency, or even nullipotency, which might be regarded as the ultimate consequence of adaptation occurring in tumours that have developed over a period of twenty or more years (Baker et al., 2007). Intriguingly, a recent report suggested that non-seminomas, most likely the yolk sac tumour component, also may have the potential to differentiate to germ cells, although this needs independent confirmation (Honecker et al., 2006). Taken together, the observations from pathology suggest that the common pre-invasive CIS cells have the capability to develop into many different germinal and somatic tissues and may be regarded as

pluripotent or truly totipotent. In that sense, we believe that CIS is a cancer stem cell for both TGCT types, seminoma and non-seminomatous tumour. However, it is debated that CIS has to be first re-programmed to EC, which then is a stem cell for somatic components of non-seminomas.

The remarkable potential of CIS cells raises the immediate question of their origin. A few years after their initial discovery, a morphological similarity to gonocytes was noted and it was proposed that CIS cells could be remnants of undifferentiated embryonic/fetal cells (Nielsen et al., 1974; Skakkebaek et al., 1987). Their fetal origin was supported by immunohistochemical studies of proteins present in CIS also shown to be present in primordial germ (PG) cells and gonocytes, and by identifying CIS cells in prepubertal patients, who later developed TGCTs, indicating that the cells had originated prior to puberty (Muller et al., 1984). Additional support was provided by epidemiological studies showing that the increase in TGCTs was related to birth cohort rather than year of diagnosis (Bergstrom et al., 1996). Remarkably, further epidemiological studies demonstrated that first-generation immigrants to Sweden had the same risk for developing TGCTs as in their country of origin, whereas second-generation immigrants had the same risk as native Swedes (Hemminki et al., 2002; Hemminki and Li, 2002). Besides strongly indicating the fetal origin of TGCTs in young adults, these epidemiological studies also showed the aetiological importance of environmental factors in development of the CIS cells, although some genetic predisposition also plays a role. Hence, TGCTs in young adults have been proposed to be part of a larger testicular dysgenesis syndrome (TDS) that includes mild genital malformations and certain forms of infertility, all thought to be highly influenced by environmental exposures (Skakkebaek et al., 2001). So, 35 years from the initial description of CIS cells, it has become increasingly clear that the attention of investigators dealing with the TGCTs and their origin has to focus on both the early *in utero* gonadal development and the impact of the multitude of different environmental factors that can negatively affect this process, including the so-called endocrine disruptors. In this review we will summarise findings concerning the phenotype and genotypic signature of CIS cells and discuss the hypotheses concerning their origin and how these embryonic cells in the adult develop into a malignant tumour.

2. Expression of embryonic marker genes in CIS cells and during invasive progression: The development of a pluripotent tumour?

CIS cells have many morphological similarities with PG cells and early gonocytes (Nielsen et al., 1974; Albrechtsen et al., 1982; Skakkebaek et al., 1987). Relatively early on it was identified that the three cell types share the expression of immunohistochemical markers such as placental-like alkaline phosphatase (PLAP) or the stem cell factor receptor KIT (Giwerzman et al., 1991; Rajpert-De Meyts and Skakkebaek, 1994; Jorgensen et al., 1995). These markers are now commonly used in the clinical setting to help identify CIS in surgical biopsies. More recently, the expression profiles of CIS cells studied by microarrays (Almstrup et al., 2004; Skotheim et al., 2005) provided a series of candidate marker genes, such as *TFAP2C* (AP2 γ) (Hoei-Hansen et al., 2004), which has shown promising results as a possible marker of CIS cells in semen samples (Hoei-Hansen et al., 2007).

A landmark in the understanding of CIS and the derived invasive cancers was the identification of *POU5F1* (OCT3/4) and *NANOG* expression, which are known for their association with pluripotency in ES cells (Figure 1) (Looijenga et al., 2003; Rajpert-De Meyts et al., 2004; Hoei-Hansen et al., 2005). These two transcription factors are, together with SOX2, key regulators of the formation and maintenance of the inner cell mass during preimplantation development and for sustaining self-renewal in ESCs (Scholer et al., 1990; Nichols et al., 1998; Niwa et al., 2000; Avilion et al., 2003; Chambers et al., 2003; Mitsui et al., 2003). Among others, knock-out studies of both *Pou5f1* and *Nanog* in mice have implicated these genes in the first and second initial fate decisions in mammalian development, respectively (Cavaleri and Scholer, 2003; Chambers et al., 2003).

Although very useful, experimental studies in animals have demonstrated differences in developmental biology between mice and humans. Stem cells have been isolated from adult mouse testes that exhibit properties similar to ES cells (Guan et al., 2006; Seandel et al., 2007), and these studies showed that a subset of spermatogonial stem cells in mouse testes retain the embryonic phenotype of PG cells and gonocytes. In humans a different scenario is seen, where NANOG and OCT3/4 (together with KIT and AP2 γ) are expressed in PG cells and gonocytes, but are down-regulated during the second half of pregnancy and until 2-4½ months of post-natal age (Jorgensen et al., 1995; Rajpert-De Meyts et al., 2004; Hoei-Hansen et al., 2004; Honecker et al., 2004; Hoei-Hansen et al., 2005). During this early infantile period, a transient increase in the production of testicular hormones occurs, known as the ‘mini-puberty’ (Forest et al., 1973), which coincides with

the final stage of differentiation of gonocytes into infantile spermatogonia (Hadziselimovic et al., 1986). Although NANOG and OCT3/4 are down-regulated, downstream factors identified in ES cells, UTF1 and REX1 (Niwa, 2001; Kristensen et al., 2005; Nishimoto et al., 2005), continue to be expressed throughout the male development in spermatogonia and are still present in the adult testes (Kristensen et al., in press). The continued expression of UTF1 and REX1, apparently uncoupled functionally from OCT3/4 and NANOG, shows that the partial embryonic phenotype of the germ cells is still evident in the mature human testes. The link between CIS cells and embryonic cells has been further supported by a study showing a substantial overlap between human ES cells and CIS cells' gene expression profiles (Almstrup et al., 2004).

There are, however, marked differences in the expression of embryonic genes among the histological types of overt tumours derived from CIS. The phenotype of seminoma is very close to that of CIS, whereas the phenotype of EC, considered the undifferentiated stem cell compartment of nonseminomas, resembles much closer that of ES cells, suggesting a re-programming or a de-differentiation event (Sperger et al., 2003; Almstrup et al., 2005; Skotheim et al., 2005; Korkola et al., 2006). This is supported by the high expression of SOX2 in EC (Perrett et al. in press), for example, while in seminoma this marker is virtually negative (Sperger et al., 2003; Santagata et al., 2007). A further somatic differentiation of EC is observed in nearly all nonseminomas and this event is associated with down-regulation/inactivation of the embryonic pluripotency genes (Figure 1, 3) (Skotheim et al., 2005; Korkola et al., 2006).

The similarities between the ES cell lines and CIS cells do not stop at gene expression patterns, there are also remarkably similar patterns of genomic aberrations in the two cell types (Figure 2). CIS cells are known to undergo polyploidization and DNA content in the triploid to hypotetraploid range has been identified in CIS adjacent to both seminoma and non-seminoma (Ottesen et al., 2004b). Although polyploidization seems to appear prior to the establishment of extra 12p material (Geurts van Kessel et al., 1989; Oosterhuis et al., 1989; Ottesen et al., 2004b), the specific stage at which tumour development is initiated and genomic instability introduced remains unresolved. Due to technical problems only a small number of cases of CIS have been examined for chromosomal aberrations, however a range of imbalances were detected, including gains of regions of the chromosomes 7, 8, 12, 14, 15, 17 and X (Looijenga et al., 2000; Summersgill et al., 2001; Ottesen et al., 2003; Ottesen et al., 2004a; Ottesen et al., 2004b). A recurrent gain of 12p material was demonstrated in the vast majority of overt TGCTs, together with chromosomal aberrations established as typical for TGCTs (Atkin & Baker 1982; Summersgill et al., 2001; and reviewed in

von Eyben, 2004). Fascinatingly, the extra 12p material was also observed in several cases of CIS adjacent to overt tumours but was absent in CIS cells with no evidence of invasive growth (Looijenga et al., 2000; Ottesen et al., 2003; Ottesen et al., 2004a; Ottesen et al., 2004b; Skotheim et al., 2006). Whereas gains of 17q were only detected in a few CIS cases (Ottesen et al., 2003; Ottesen et al., 2004b), a recurrent gain of 17q has been associated with non-seminomas in particular, perhaps illustrating the high proliferation potential of this more aggressive tumour (Kraggerud et al., 2002; Skotheim et al., 2002).

Gains in largely the same chromosomal regions are seen in cultured ES cells after prolonged culture indicating that the microenvironment may play a role in increasing genomic instability (Draper et al., 2004; Andrews et al., 2005; Baker et al., 2007). Evidence suggests that the ES cells ‘adapt’ through these genomic gains, proliferating faster and becoming easier to maintain with time in culture. In addition, when cells from such an “adapted” culture were inoculated into a SCID (severe combined immunodeficient) mouse, a teratocarcinoma containing histologically recognisable stem cells was formed, which grew out when the tumour was explanted into culture and exhibited properties of the starting ES cells (Andrews et al., 2005). Therefore, the “adapted” ES cells resembled the malignant non-seminoma EC cells. As such, ES cells show adaptive changes during culture which mimic changes occurring in CIS and later the derived EC cells during tumour progression. Thus, the non-random genomic aberrations seen in CIS cells may be caused by selection of cells with a proliferative advantage during tumour development and progression (Figure 3), for example those with an over-expression of the pluripotency-related genes located on the short arm of chromosome 12 (Clark et al., 2004; Korkola et al., 2006). The simplistic hypothesis would be that only a few genes are involved, perhaps only one from each region amplified. However, the situation may be more complex, which is supported by the historical difficulty in identifying single genes that underlie common chromosome amplifications in various cancers, not only TGCTs (Baker et al., 2007). The fact that the microenvironment plays such a defining role in genomic instability in ES cells could indicate that the same is true during the formation of CIS cells. It is quite possible that a disturbance or disruption of the original niche of gonocytes or PG cells may have led to imbalance between proliferation and differentiation resulting in the genetic instability and neoplastic transformation. As time passes, the CIS cells also develop through selection, eventually resulting in the progression to a malignant seminoma or non-seminoma.

3. The CIS cancer stem cells

The pathogenesis of TGCTs in young men has not yet been elucidated in detail. However, it is obvious that unravelling the biology of CIS cells is fundamental for the understanding of the aetiology of TGCTs. The CIS cell represents an interesting variant of cancer stem cell since it originates before the tissue that it propagates in is fully differentiated and functional. The concept that cancers arise from ‘stem cells’ or ‘germ cells’ was already proposed about 150 years ago and recently, due to advances in stem cell biology, has been given a revival with the proposal of the ‘cancer stem cell hypothesis’ (reviewed in Wicha et al., 2006). The two related concepts of this hypothesis are that (i) tumours originate in either tissue stem cells or their immediate progeny through malfunctions in the process of self-renewal. As a result of this, (ii) tumours contain so-called ‘cancer stem cells’ as a cellular subcomponent that retains key stem cell properties. These properties include self-renewal, which drives tumourigenesis, and differentiation that contributes to the cellular heterogeneity seen in most cancers. However, in contrast to TGCTs, the cellular heterogeneity is limited to the same tissue type in the vast majority of somatic cancers. Cancer stem cells are thought to function in a similar way as normal epithelial tissue stem cells that constantly replenish a tissue with committed differentiated cells not capable of self-renewal. Within the last couple of years experimental evidence in a variety of tumours has lent support to the cancer stem cell hypothesis that represents a paradigm shift in the understanding of carcinogenesis and tumour cell biology (Wicha et al., 2006).

The initiation of the neoplastic transformation generating CIS cells, which is discussed in detail below, is most likely triggered by a disturbance in the embryonic, fetal, or – perhaps in some cases – also early postnatal microenvironment of differentiating germ cells. Thus, CIS cells are different from the “usual” somatic cancer stem cells due to their origin in early development long before the tissue is fully differentiated and also in their ability to give rise to tumours with pluripotent potential. Regardless of these differences, CIS cells should be regarded as genuine cancer stem cells. The cells self-renew and are often found in the periphery of these solid tumours. The CIS cells are malignant cells that have the ability to invade all tubules in the testis and form solid seminomas tumours. In another cases, which tend to be relatively younger men than those who develop seminomas, CIS cells undergo a further development and give rise to non-seminomas, which include a variety of histologically different invasive tumours. This was already shown in the first description of the cells in 1972, where two patients with CIS were followed to the point where they developed invasive tumours (Skakkebaek, 1972b; Skakkebaek, 1978). Furthermore, later

studies showed that patients with CIS, which seemed cured after chemotherapy, developed cancer after the treatment (Maase et al., 1988; Dieckmann, 1988; Dieckmann and Loy, 1991). This indicates that the cells have the capacity to repopulate the testis from a single or only few cells, which is a classic characteristic of cancer stem cells. Still, it is unresolved to what degree CIS cells make the transition to the invasive tumours and whether all CIS cells are destined to become invasive. It seems likely that only one or a few cells at a time make the transition to develop into an invasive cancer. However, testes with both seminoma and non-seminoma tumours separated by a non-malignant tissue are not uncommon, indicating a possible polyclonal origin of these tumours from at least two CIS cells.

Much remains unknown regarding the formation of solid tumours from CIS cells, especially the development of non-seminomas. The development of CIS cells to EC is particularly intriguing since it seems to encompass more than just a classic differentiation, and resembles a de-differentiation to a more original embryonic cell, mimicking cells from the inner cell mass of the early embryo.

4. Does disturbance of the stem cell niche result in development of CIS cells? The possible role of endocrine disrupters.

The differentiating cells in the child are very susceptible to environmental damage, because development is a highly integrated process in which massive proliferation and extensive differentiation are synchronized with each other and with apoptosis (Birnbaum and Fenton, 2003). The extensive growth rates allow for mutagenic and epigenetic alterations. Likewise, differentiation represents a controlled process in which patterns of gene expression undergo large changes. Consequently, both cell division and differentiation offer multiple opportunities for the initiation of lesions as well as the promotion of the growth of altered cells initiating the complex process of developing a cancer. Moreover, physiological barriers protective of external factors such as the blood-brain barrier and blood-testes barrier are not complete *in utero*. Furthermore, the metabolising and elimination capabilities of the developing organism are not fully developed until after birth, leaving the fetus and newborn particularly susceptible to the adverse effects of environmental compounds (Birnbaum and Fenton, 2003).

Several recent studies have elucidated the mechanism of early differentiation of sexually bipotential primordial germ cells into the male cell lineage, however, much deeper insight into the early human embryogenesis is needed to understand the development of CIS cells. Rare genetic

disorders of sex differentiation have yielded insight into the possible mechanisms of CIS cell formation. For example, in some individuals with mutations in *SRY* and 46,XY gonadal dysgenesis, CIS cells and/or gonadoblastoma may be seen in the streak or in severely dysgenetic streak gonads (Muller et al., 1992; Cools et al., 2006). In addition to rare instances of a mutation in *SRY*, a more commonly observed lack of the Y chromosome in a subset of cells in the developing testis (a mosaic sex chromosome aneuploidy) would cause a focal lack of masculinisation and a varying degree of gonad dysgenesis, often with the occurrence of gonadoblastoma or CIS. However, in the vast majority of cases of sporadic CIS/TGCT in adult men the signs of dysgenesis are very discreet but nearly always consistent with focal lack of proper virilization and gonad maturation, manifested by clusters of distorted tubules and undifferentiated Sertoli cells (Hoei-Hansen et al., 2003; Nistal et al., 2006). These studies combined with lessons from disorders of sex differentiation and epidemiological observations support the notion that interference with early male development, either from genetic mutation or environmental factors, can lead to the development of CIS cells and hence neoplasia.

The differentiation of human gonocytes into infantile spermatogonia spans a relatively long period with a gradual down-regulation of the embryonic pluripotency genes and up-regulation of germ cell-specific genes with a role in spermatogenesis. Analysis of the CIS phenotype and the presence of CIS cells in certain conditions associated with testicular dysgenesis have provided strong evidence that developmental arrest in the differentiation of the early germ cell lineage is a core pathogenetic event leading to neoplastic transformation into CIS (Figure 3) (Rajpert-De Meyts et al., 1996; Rajpert-De Meyts et al., 1998; Rajpert-De Meyts, 2006). Thus, it seems plausible that the long duration of differentiation leaves a window for disturbances in the hormonal balance that may topple the delicate balance and delay or arrest the differentiation of PG cells or gonocytes into infantile pre-spermatogonia. Moreover, the differentiation of germ cells occurs in an intimate contact with somatic cells in the microcellular “niche”, which consists of Sertoli cells, Leydig cells, and peritubular myoid cells, and it is in these few cell types that hormonal and paracrine factors responsible for the differentiation process are produced. These cells are therefore the likely targets of environmental factors such as endocrine disrupters, which is supported by a clear association of germ cell neoplasia with insufficient function of the androgen-producing Leydig cells, and with decreased proliferation of Sertoli cells in association with small testes with reduced spermatogenesis (Holm et al., 2003). Problems with poorly developed or partially hyalinised tubule walls are also a common feature of CIS tubules. All these features can be reproduced in

experimental animal models by exposure to selected endocrine disruptors, in particular various anti-androgenic compounds, including phthalates (Mylchreest et al., 1998; Fisher et al., 2003; Sharpe, 2006). There is still only indirect evidence from human studies, but the association of testicular cancer with exposures to some of these compounds has already been suggested by several studies, indicating that the development of CIS cells could be directly coupled to endocrine disruptors disturbing early male development (Hardell et al., 2003; Swan et al., 2005; Main et al., 2007).

5. Concluding Remarks

The study of CIS cells has its focus on two major events: (i) the initial development *in utero* of the CIS cell and (ii) the progression of CIS cells in adulthood to an invasive malignant tumour. There is growing evidence that disturbances of the cellular microenvironment of developing germ cells, also known as their “niche”, are of a primary importance in the development of the CIS cells and also in the origin of other disorders of testicular function, even those manifested in adulthood. As these disorders are becoming increasingly common, we must consider the negative impact of the modern lifestyle and strive to find the responsible factors together with the pathways they target, especially during early human embryogenesis. Numerous studies have demonstrated that germ cell tumours derived from CIS cells mimic embryonic development and constitute a model to study pathways of pluripotent cell differentiation. In this way germ cell tumours and ES cells are highly similar and it has been shown that during extended culture human ES cells recapitulate features of the malignant transformation seen in germ cell tumours. Hence, the knowledge obtained from studies of human ES cells *in vitro* can help to understand the mechanisms of neoplastic transformation of germ cells and invasive progression of tumours.

Acknowledgements

The authors acknowledge the generous financial support of the Danish Cancer Society, the Svend Andersen Foundation and the Vissing Foundation.

References

- Albrechtsen, R., Nielsen, M.H., Skakkebaek, N.E., Wewer, U., 1982. Carcinoma in situ of the testis. Some ultrastructural characteristics of germ cells. *Acta Pathol. Microbiol. Immunol. Scand.* 90, 301-303.
- Almstrup, K., Hoei-Hansen, C.E., Nielsen, J.E., Wirkner, U., Ansorge, W., Skakkebaek, N.E., Rajpert-De Meyts, E., and Leffers, H., 2005. Genome-wide gene expression profiling of testicular carcinoma in situ progression into overt tumours. *Br. J. Cancer.* 92, 1934-1941.
- Almstrup, K., Hoei-Hansen, C.E., Wirkner, U., Blake, J., Schwager, C., Ansorge, W., Nielsen, J.E., Skakkebaek, N.E., Rajpert-De Meyts, E., Leffers, H., 2004. Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. *Cancer Res.* 64, 4736-4743.
- Andrews, P.W., 1998. Teratocarcinomas and human embryology: pluripotent human EC cell lines. Review article. *APMIS.* 106, 158-167.
- Andrews, P.W., Matin, M.M., Bahrami, A.R., Damjanov, I., Gokhale, P., Draper, J.S., 2005. Embryonic stem (ES) cells and embryonal carcinoma (EC) cells: opposite sides of the same coin. *Biochem. Soc. Trans.* 33, 1526-1530.
- Avilion, A.A., Nicolis, S.K., Pevny, L.H., Perez, L., Vivian, N., Lovell-Badge, R. 2003. Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.* 17, 126-140.
- Baker, D.E., Harrison, N.J., Maltby, E., Smith, K., Moore, H.D., Shaw, P.J., Heath, P.R., Holden, H., Andrews, P.W., 2007. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. *Nat. Biotechnol.* 25, 207-215.
- Bergstrom, R., Adami, H.O., Mohnner, M., Zatonski, W., Storm, H., Ekbom, A., Tretli, S., Teppo, L., Akre, O., Hakulinen, T., 1996. Increase in testicular cancer incidence in six European countries: a birth cohort phenomenon. *J. Natl. Cancer Inst.* 88, 727-733.
- Birnbaum, L.S. and Fenton, S.E., 2003. Cancer and developmental exposure to endocrine disruptors. *Environ. Health Perspect.* 111, 389-394.
- Cavaleri, F. and Scholer, H.R., 2003. Nanog: a new recruit to the embryonic stem cell orchestra. *Cell.* 113, 551-552.
- Chambers, I., Colby, D., Robertson, M., Nichols, J., Lee, S., Tweedie, S., Smith, A., 2003. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 113, 643-655.
- Clark, A.T., Rodriguez, R.T., Bodnar, M.S., Abeyta, M.J., Cedars, M.I., Turek, P.J., Firpo, M.T., Reijo-Pera, R.A., 2004. Human STELLAR, NANOG, and GDF3 genes are expressed in pluripotent cells and map to chromosome 12p13, a hotspot for teratocarcinoma. *Stem Cells* 22, 169-179.
- Cools, M., Stoop, H., Kersemaekers, A.M., Drop, S.L., Wolffenbuttel, K.P., Bourguignon, J.P., Slowikowska-Hilczek, J., Kula, K., Faradz, S.M., Oosterhuis, J.W., Looijenga, L.H., 2006.

Gonadoblastoma arising in undifferentiated gonadal tissue within dysgenetic gonads. *J. Clin. Endocrinol. Metab.* 91, 2404-2413.

Dieckmann, K.P., 1988. Residual carcinoma-in-situ of contralateral testis after chemotherapy. *Lancet.* 1, 765.

Dieckmann, K.P. and Loy, V., 1991. Persistent testicular intraepithelial neoplasia after chemotherapy. *Eur. Urol.* 20, 258-260.

Draper, J.S., Smith, K., Gokhale, P., Moore, H.D., Maltby, E., Johnson, J., Meisner, L., Zwaka, T.P., Thomson, J.A., Andrews, P.W., 2004. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat. Biotechnol.* 22, 53-54.

von Eyben, F.E., 2004. Chromosomes, genes, and development of testicular germ cell tumors. *Cancer Genet. Cytogenet.* 151, 93-138.

Fisher, J.S., Macpherson, S., Marchetti, N., Sharpe, R.M., 2003. Human 'testicular dysgenesis syndrome': a possible model using in-utero exposure of the rat to dibutyl phthalate. *Hum. Reprod.* 18, 1383-1394.

Forest, M.G., Cathiard, A.M., Bertrand, J.A., 1973. Evidence of testicular activity in early infancy. *J. Clin. Endocrinol. Metab.* 37, 148-151.

Geurts van Kessel, A., van Drunen, E., de Jong, B., Oosterhuis, J.W., Langeveld, A., Mulder, M.P., 1989. Chromosome 12q heterozygosity is retained in i(12p)-positive testicular germ cell tumor cells. *Cancer Genet. Cytogenet.* 40, 129-134.

Giwerzman, A., Cantell, L., Marks, A. 1991. Placental-like alkaline phosphatase as a marker of carcinoma-in-situ of the testis. Comparison with monoclonal antibodies M2A and 43-9F. *APMIS.* 99, 586-594.

Guan, K., Nayernia, K., Maier, L.S., Wagner, S., Dressel, R., Lee, J.H., Nolte, J., Wolf, F., Li, M., Engel, W., Hasenfuss, G., 2006. Pluripotency of spermatogonial stem cells from adult mouse testis. *Nature.* 440, 1199-1203.

Hadziselimovic, F., Thommen, L., Girard, J., Herzog, B., 1986. The significance of postnatal gonadotropin surge for testicular development in normal and cryptorchid testes. *J. Urol.* 136, 274-276.

Hardell, L., van Bavel, B., Lindstrom, G., Carlberg, M., Dreifaldt, A.C., Wijkstrom, H., Starkhammar, H., Eriksson, M., Hallquist, A., Kolmert, T., 2003. Increased concentrations of polychlorinated biphenyls, hexachlorobenzene, and chlordanes in mothers of men with testicular cancer. *Environ. Health Perspect.* 111, 930-934.

Hemminki, K., Li, X., 2002. Cancer risks in second-generation immigrants to Sweden. *Int. J. Cancer.* 99, 229-237.

Hemminki, K., Li, X., Czene, K., 2002. Cancer risks in first-generation immigrants to Sweden. *Int. J. Cancer.* 99, 218-228.

- Hoei-Hansen, C.E., Holm, M., Rajpert-De Meyts, E., Skakkebaek, N.E., 2003. Histological evidence of testicular dysgenesis in contralateral biopsies of 218 patients with testicular germ cell cancer. *J Pathol*; 200, 370-374.
- Hoei-Hansen, C.E., Almstrup, K., Nielsen, J.E., Brask, S.S., Graem, N., Skakkebaek, N.E., Leffers, H., Rajpert-De Meyts, E., 2005. Stem cell pluripotency factor NANOG is expressed in human fetal gonocytes, testicular carcinoma in situ and germ cell tumours. *Histopathology*. 47, 48-56.
- Hoei-Hansen, C.E., Carlsen, E., Jorgensen, N., Leffers, H., Skakkebaek, N.E., Rajpert-De Meyts, E., 2007. Towards a non-invasive method for early detection of testicular neoplasia in semen samples by identification of fetal germ cell-specific markers. *Hum. Reprod*. 22, 167-173.
- Hoei-Hansen, C.E., Nielsen, J.E., Almstrup, K., Sonne, S.B., Graem, N., Skakkebaek, N.E., Leffers, H., Rajpert-De Meyts, E., 2004. Transcription factor AP-2gamma is a developmentally regulated marker of testicular carcinoma in situ and germ cell tumors. *Clin. Cancer Res*. 10, 8521-8530.
- Holm, M., Rajpert-De Meyts, E., Andersson, A.M., Skakkebaek, N.E., 2003. Leydig cell micronodules are a common finding in testicular biopsies from men with impaired spermatogenesis and are associated with decreased testosterone/LH ratio. *J. Pathol*. 199, 378-386.
- Honecker, F., Stoop, H., de Krijger, R.R., Lau, Y.-F.C., Bokemeyer, C., Looijenga, L.H., 2004. Pathobiological implications of the expression of markers of testicular carcinoma in situ by fetal germ cells. *J. Pathol*. 203, 849-857.
- Honecker, F., Stoop, H., Mayer, F., Bokemeyer, C., Castrillon, D.H., Lau, Y.F., Looijenga, L.H., Oosterhuis, J.W., 2006. Germ cell lineage differentiation in non-seminomatous germ cell tumours. *J. Pathol*. 208, 395-400.
- Jorgensen, N., Rajpert-De Meyts, E., Graem, N., Muller, J., Giwercman, A., Skakkebaek, N.E., 1995. Expression of immunohistochemical markers for testicular carcinoma in situ by normal human fetal germ cells. *Lab Invest*. 72, 223-231.
- Korkola, J.E., Houldsworth, J., Chadalavada, R.S., Olshen, A.B., Dobrzynski, D., Reuter, V.E., Bosl, G.J., 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 Research* 66, 820-827.
- Kraggerud, S.M., Skotheim, R.I., Szymanska, J., Eknaes, M., Fossa, S.D., Stenwig, A.E., Peltomaki, P., Lothe, R.A., 2002. Genome profiles of familial/bilateral and sporadic testicular germ cell tumors. *Genes Chromosomes. Cancer*. 34, 168-174.
- Kristensen, D.M., Kalisz, M., Nielsen, J.H., 2005. Cytokine signalling in embryonic stem cells. *APMIS*. 113, 756-772.
- Kristensen, D.M., Nielsen, J.E., Skakkebaek N.E., Graem N., Jacobsen G.K., Rajpert-De Meyts, E., Leffers H., 2008. Presumed pluripotency markers UTF-1 and REX-1 are expressed in human adult testes and germ cell neoplasms. *Hum Reprod. In press*.

- Looijenga, L.H., Rosenberg, C., van Gurp, R.J., Geelen, E., Echten-Arends, J., de Jong, B., Mostert, M., Wolter, O.J., 2000. Comparative genomic hybridization of microdissected samples from different stages in the development of a seminoma and a non-seminoma. *J. Pathol.* 191, 187-192.
- Looijenga, L.H., Stoop, H., de Leeuw, H.P., Gouveia Brazao, C.A., Gillis, A.J., van Roozendaal, K.E., van Zoelen, E.J., Weber, R.F., Wolffenbuttel, K.P., van Dekken, H., Honecker, F., Bokemeyer, C., Perlman, E.J., Schneider, D.T., Kononen, J., Sauter, G., Oosterhuis, J.W., 2003. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Res.* 63, 2244-2250.
- Maase, v.d.H., Meinecke, B., Skakkebaek, N.E., 1988. Residual carcinoma-in-situ of contralateral testis after chemotherapy. *Lancet.* 27, 477-478.
- Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J., Vartiainen, T., Skakkebaek, N.E., Toppari, J., 2007. Flame Retardants in Placenta and Breast Milk and Cryptorchidism in Newborn Boys. *Environ. Health Perspect.* 115, 1519-1526.
- Mitsui, K., Tokuzawa, Y., Itoh, H., Segawa, K., Murakami, M., Takahashi, K., Maruyama, M., Maeda, M., Yamanaka, S., 2003. The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells. *Cell.* 113, 631-642.
- Muller, J., Skakkebaek, N.E., Nielsen, O.H., Graem, N., 1984. Cryptorchidism and testis cancer. Atypical infantile germ cells followed by carcinoma in situ and invasive carcinoma in adulthood. *Cancer.* 54, 629-634.
- Muller, J., Visfeldt, J., Philip, J., Skakkebaek, N.E., 1992. Carcinoma in situ, gonadoblastoma, and early invasive neoplasia in a nine-year-old girl with 46,XY gonadal dysgenesis. *APMIS.* 100, 170-174.
- Mylchreest, E., Cattley, R.C., Foster, P.M., 1998. Male reproductive tract malformations in rats following gestational and lactational exposure to Di(n-butyl) phthalate: an antiandrogenic mechanism? *Toxicol Sci* 43, 47-60.
- Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H., Smith, A., 1998. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* 95, 379-391.
- Nielsen, H., Nielsen, M., Skakkebaek, N.E., 1974. The fine structure of possible carcinoma-in-situ in the seminiferous tubules in the testis of four infertile men. *Acta Pathol. Microbiol. Scand. [A].* 82, 235-248.
- Nishimoto, M., Miyagi, S., Yamagishi, T., Sakaguchi, T., Niwa, H., Muramatsu, M., Okuda, A., 2005. Oct-3/4 maintains the proliferative embryonic stem cell state via specific binding to a variant octamer sequence in the regulatory region of the UTF1 locus. *Mol. Cell Biol.* 25, 5084-5094.
- Nistal, M., Gonzalez-Peramato, P., Regadera, J., Serrano, A., Tarin, V., De Miguel, M.P., 2006. Primary testicular lesions are associated with testicular germ cell tumors of adult men. *Am. J. Surg. Pathol.* 30, 1260-1268.

Niwa, H., 2001. Molecular mechanism to maintain stem cell renewal of ES cells. *Cell Struct. Funct.* 26, 137-148.

Niwa, H., Miyazaki, J., Smith, A.G., 2000. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* 24, 372-376.

Oosterhuis, J.W., Castedo, S.M., de Jong, B., Seruca, R., Dam, A., Vos, A., de Koning, J., Schraffordt, K.H., Sleijfer, D.T., 1989. A malignant mixed gonadal stromal tumor of the testis with heterologous components and i(12p) in one of its metastases. *Cancer Genet. Cytogenet.* 41, 105-114.

Oosterhuis, J.W., Looijenga, L.H., 2005. Testicular germ-cell tumours in a broader perspective. *Nat. Rev. Cancer.* 5, 210-222.

Ottesen, A.M., Larsen, J., Gerdes, T., Larsen, J.K., Lundsteen, C., Skakkebaek, N.E., Rajpert-De Meyts, E., 2004a. Cytogenetic investigation of testicular carcinoma in situ and early seminoma by high-resolution comparative genomic hybridization analysis of subpopulations flow sorted according to DNA content. *Cancer Genet. Cytogenet.* 149, 89-97.

Ottesen, A.M., Rajpert-De Meyts, E., Holm, M., Andersen, I.L., Vogt, P.H., Lundsteen, C., Skakkebaek, N.E., 2004b. Cytogenetic and molecular analysis of a family with three brothers afflicted with germ-cell cancer. *Clin. Genet.* 65, 32-39.

Ottesen, A.M., Skakkebaek, N.E., Lundsteen, C., Leffers, H., Larsen, J., Rajpert-De Meyts, E., 2003. High-resolution comparative genomic hybridization detects extra chromosome arm 12p material in most cases of carcinoma in situ adjacent to overt germ cell tumors, but not before the invasive tumor development. *Genes Chromosomes. Cancer.* 38, 117-125.

Perrett R.M., Turpenney L., Eckert J.J., O'Shea M., Sonne S.B., Cameron I.T., Wilson D.A., Rajpert-De Meyts E., Hanley N.A., 2008. The human germ cell lineage does not express SOX2 during early development or in early *in vitro* culture. *Biol Reprod. In press.*

Rajpert-De Meyts, E., 2006. Developmental model for the pathogenesis of testicular carcinoma in situ: genetic and environmental aspects. *Hum. Reprod. Update.* 12, 303-323.

Rajpert-De Meyts, E., Hanstein, R., Jorgensen, N., Graem, N., Vogt, P.H., Skakkebaek, N.E., 2004. Developmental expression of POU5F1 (OCT-3/4) in normal and dysgenetic human gonads. *Hum. Reprod.* 19, 1338-1344.

Rajpert-De Meyts, E., Jorgensen, N., Brøndum-Nielsen, K., Müller, J., Skakkebaek, N.E., 1998. Developmental arrest of germ cells in the pathogenesis of germ cell neoplasia. *APMIS* 106, 198-206.

Rajpert-De Meyts, E., Jørgensen, N., Müller, J., Skakkebaek, N.E., 1996. Prolonged expression of the c-kit receptor in germ cells of intersex fetal testes. *J. Pathol.* 178, 166-169.

Rajpert-De Meyts, E., Skakkebaek, N.E., 1994. Expression of the c-kit protein product in carcinoma-in-situ and invasive testicular germ cell tumours. *Int. J. Androl.* 17, 85-92.

- Richiardi, L., Bellocco, R., Adami, H.O., Torrang, A., Barlow, L., Hakulinen, T., Rahu, M., Stengrevics, A., Storm, H., Tretli, S., Kurtinaitis, J., Tyczynski, J.E., Akre, O., 2004. Testicular cancer incidence in eight northern European countries: secular and recent trends. *Cancer Epidemiol. Biomarkers Prev.* 13, 2157-2166.
- Santagata, S., Ligon, K.L., 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.
- Scholer, H.R., Ruppert, S., Suzuki, N., Chowdhury, K., Gruss, P., 1990. New type of POU domain in germ line-specific protein Oct-4. *Nature.* 344, 435-439.
- Seandel, M., James, D., Shmelkov, S.V., Falciatori, I., Kim, J., Chavala, S., Scherr, D.S., Zhang, F., Torres, R., Gale, N.W., Yancopoulos, G.D., Murphy, A., Valenzuela, D.M., Hobbs, R.M., Pandolfi, P.P., Rafii, S., 2007. Generation of functional multipotent adult stem cells from GPR125+ germline progenitors. *Nature.* 20, 449, 346-350.
- Sharpe, R.M., 2006. Pathways of endocrine disruption during male sexual differentiation and masculinization. *Best. Pract. Res. Clin. Endocrinol. Metab.* 20, 91-110.
- Skakkebaek, N.E., 1972a. Abnormal morphology of germ cells in two infertile men. *Acta Pathol. Microbiol. Scand. [A].* 80, 374-378.
- Skakkebaek, N.E., 1972b. Possible carcinoma-in-situ of the testis. *Lancet.* 2, 516-517.
- Skakkebaek, N.E., 1978. Carcinoma in situ of the testis: frequency and relationship to invasive germ cell tumours in infertile men. *Histopathology.* 2, 157-170.
- Skakkebaek, N.E., Berthelsen, J.G., Giwercman, A., Muller, J., 1987. Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. *Int. J. Androl.* 10, 19-28.
- Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M., 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum. Reprod.* 16, 972-978.
- Skotheim, R.I., Autio, R., Lind, G.E., Kraggerud, S.M., Andrews, P.W., Monni, O., Kallioniemi, O., Lothe, R.A., 2006. Novel genomic aberrations in testicular germ cell tumors by array-CGH, and associated gene expression changes. *Cell Oncol.* 28, 315-326.
- Skotheim, R.I., Lind, G.E., Monni, O., Nesland, J.M., Abeler, V.M., Fossa, S.D., Duale, N., Brunborg, G., Kallioniemi, O., Andrews, P.W., Lothe, R.A., 2005. Differentiation of human embryonal carcinomas in vitro and in vivo reveals expression profiles relevant to normal development. *Cancer Res.* 65, 5588-5598.
- Skotheim, R.I., Monni, O., Mousses, S., Fossa, S.D., Kallioniemi, O.P., Lothe, R.A., Kallioniemi, A., 2002. New insights into testicular germ cell tumorigenesis from gene expression profiling. *Cancer Res.* 62, 2359-2364.
- Solter, D., 2006. From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research. *Nat. Rev. Genet.* 7, 319-327.

Sperger, J.M., Chen, X., Draper, J.S., Antosiewicz, J.E., Chon, C.H., Jones, S.B., Brooks, J.D., Andrews, P.W., Brown, P.O., Thomson, J.A., 2003. Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13350-13355.

Summersgill, B., Osin, P., Lu, Y.J., Huddart, R., Shipley, J., 2001. Chromosomal imbalances associated with carcinoma in situ and associated testicular germ cell tumours of adolescents and adults. *Br. J. Cancer.* 85, 213-220.

Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ. Health Perspect.* 113, 1056-1061.

Ulbright, T.M., Amin, M.B., Young, R.H., 1999. Atlas of tumor pathology. Tumors of the testis, adnexa, spermatic cord, and scrotum. (Washington DC, USA: Armed Forces Institute of Pathology), pp. 1-385.

Visfeldt, J., Jorgensen, N., Muller, J., Moller, H., Skakkebaek, N.E., 1994. Testicular germ cell tumours of childhood in Denmark, 1943-1989: incidence and evaluation of histology using immunohistochemical techniques. *J. Pathol.* 174, 39-47.

Wicha, M.S., Liu, S., Dontu, G., 2006. Cancer stem cells: an old idea--a paradigm shift. *Cancer Res.* 66, 1883-1890.

Figure legends:**Figure 1:**

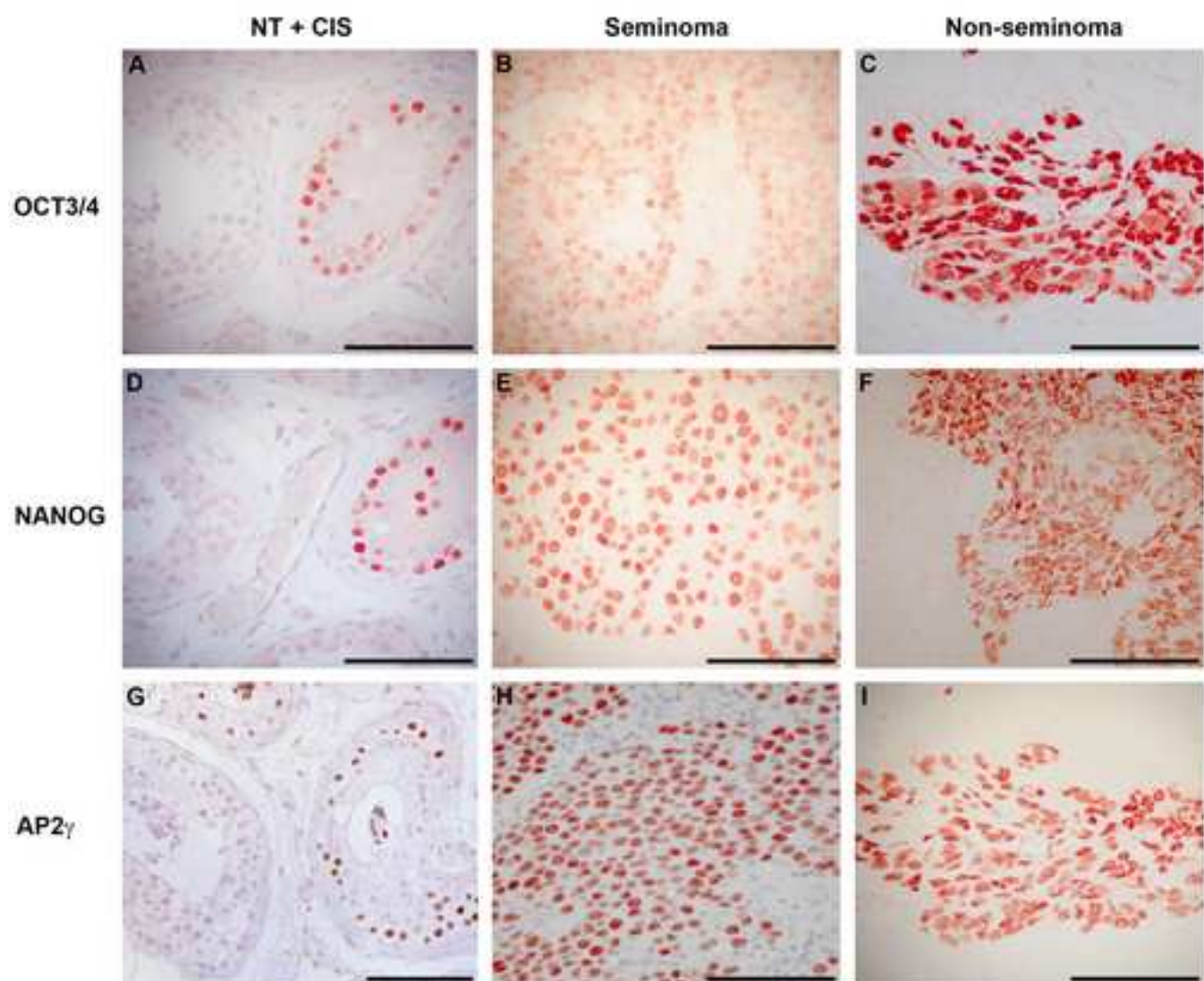
Expression of pluripotency associated proteins OCT4, NANOG, and AP2 γ in carcinoma *in situ* (CIS) cells and testicular germ cell tumours. Images A, D, and G show areas of normal testis (NT, unstained) adjacent to tubules with CIS cells (stained positively for OCT4, NANOG, and AP2 γ). Images B, E, and H show examples of classical seminoma strongly positive for the same transcription factors. Images C, F and I show the expression of the three proteins in non-seminomas; note the intensely positive clusters of embryonal carcinoma (EC), whereas the surrounding teratomatous tissues or atrophic tubules without malignancy (in F) show no staining (scale bar 100 μ m).

Figure 2:

Schematic figure showing relative gains in regions of chromosomes (chr) 12, 17, and X detected in three studies analysing CIS adjacent to overt tumours. On the right side of the chromosomes red, blue, and green bars show the extension of the gains observed in study 1, 2, and 3, respectively. Study 1: Looijenga et al., 2000; study 2: Ottesen et al., 2003; study 3: Summersgill et al., 2001.

Figure 3:

A model of the origin of carcinoma *in situ* (CIS) of the testis in early development and hypothetical pathways of progression to overt testicular germ cell tumours (TGCTs). The key events in the origin and progression of TGCT are represented in red: (1) The arrest of differentiating primordial germ cells or gonocytes and (2) the increased invasiveness with (in embryonal carcinoma, EC) or without (in seminoma) loss of germ cell phenotype. In the case of non-seminomas, EC is the presumed stem cell compartment that may undergo down-regulation of the pluripotency genes and differentiate to somatic non-seminomatous components such as teratoma (TER), yolk sac tumour (YSC) or choriocarcinoma (CHC) (modified from Rajpert-De Meyts, 2006).



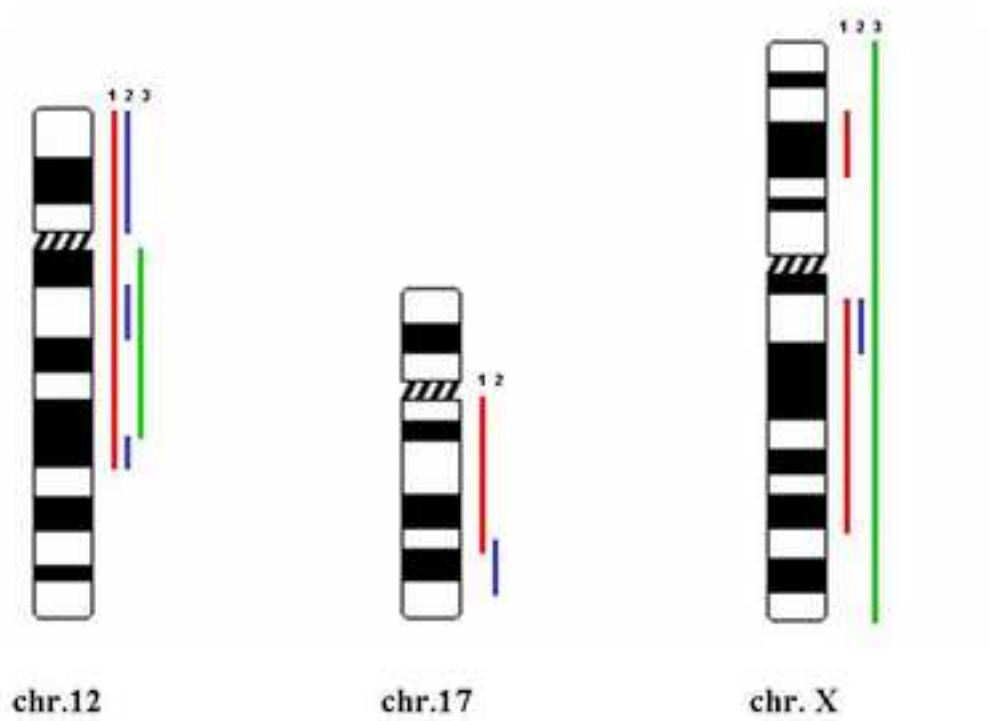


Figure 3

