

Carcinoma *in situ* -from clinical observation to a paradigm shift for testicular carcinogenesis

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ABSTRACT Carcinoma *in situ* in the testis has its origin in the fetal gonad and leads to testicular cancer in young adulthood. It can be detected in a testicular biopsy and treated with irradiation, which can preserve testosterone production of the man. These are the key findings of Niels E. Skakkebaek that changed our view of testicular tumourigenesis during the last fifty years. These and other findings are discussed in this interview-based review of testicular germ cell cancer.

KEY WORDS: *testis, cancer, fertility, CIS, germ cell*

I have had the privilege to collaborate with Niels E. Skakkebaek (Fig. 1) on testis research for a long time. Over the years we have often discussed the development of science and particularly progress in testicular cancer research. I will here describe the exciting history how the recognition of carcinoma *in situ* of the testis led to a paradigm shift in our understanding of testicular tumourigenesis and subsequent changes in management of patients at risk of testicular germ cell cancer.

As a young medical doctor Niels E. Skakkebaek started a research project on meiotic chromosomes in infertile men. He soon realized that good histopathology and microscopy of the testicular structure were essential for this work and he looked for the best possible teacher of testicular histology. Professor Yves Clermont at McGill University in Montreal had characterized both human and rodent spermatogenic cells in detail, and it was in his laboratory where Skakkebaek got a short but intensive training in morphology of germ cells. This knowledge was instrumental when he examined testicular biopsies from the infertile men. He recognized abnormal germ cells in testicular sections of two infertile men, details of which were published in APMIS (Skakkebaek, 1972). He wanted to perform further studies using EM technique on these two cases and by the time of second biopsy some years after the first one had been obtained both men had developed embryonal carcinoma and the abnormal germ cells were also found in the areas around the tumour. This finding gave Skakkebaek the idea that the abnormal cells in the first biopsy of both patients possibly had been precursor cells of testicular germ cell cancer and Skakkebaek therefore called the abnormal cell pattern carcinoma *in situ* (CIS) of the testis. The hypothesis was published in a paper in The Lancet in 1972 (Skakkebaek, 1972b), where he also presented

evidence that the CIS cells were hyperdiploid. Electron microscopic investigations which followed confirmed that the CIS cells were different from spermatogenic cells and similar to primordial germ cells or gonocytes (Nielsen *et al.*, 1974). He then turned to archive specimens of both seminomas and non-seminomas and found CIS like tubules in the adjacent 'normal' testicular tissue of both seminomas and non-seminomas (Skakkebaek, 1975). Still, urologists and oncologists were not completely convinced that these cells were pre-invasive and malignant. However, a prospective study of patients with CIS detected by chance during infertility-workup showed that some developed seminomas, others non-seminomas (Skakkebaek, 1978) and Skakkebaek was now convinced that seminomas and non-seminomas had a common origin from CIS cells. To convince pathologists and urologists he organized together with Jakob Visfeldt and Kenneth Grigor, two pathologists, a workshop in 1980 with both practical microscopy sessions and lectures on CIS and testis cancer, all published in the proceedings Early Detection of Testicular Cancer (Fig. 2). Prominent testis cancer researchers participated. The workshop which was reported in an editorial in the Lancet convinced most people, but not all, that CIS was a precursor of both non-seminomas and seminomas. However, several pathologists in the USA remained sceptical for many years. A reason might be that atypical germ cells around testicular tumours had been noticed and also reported now and then by pathologists and not considered significant, most often just assumed to be effects of tumour. However, by the time another Copenhagen workshop was arranged in 1986 by Skakkebaek's

Abbreviations used in this paper: CIS, carcinoma *in situ*.

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Fig. 1. Niels E. Skakkebaek was born in 1936 in Vodskov (Ålborg), Denmark. He graduated from the Medical School, University of Copenhagen in 1965, and received his Doctor of Medical Sciences degree on Studies on meiotic chromosomes in infertile males in 1974. He served as the Professor of Pediatrics, University of Copenhagen, in Fuglebakken Children's Hospital and Hvidovre Hospital 1982 - 1990. He founded the Department of Growth and Reproduction in University of Copenhagen, Rigshospitalet, where he worked as the Professor and Chairman 1990-2006. He continues his work there as a research professor.

colleague in Oncology Department, Mikael Rørth, the CIS theory had been accepted by most people working in the field. By that time enough evidence had been collected to launch the idea that CIS originated from fetal germ cells (Skakkebaek *et al.*, 1987), a hypothesis that has then been tested in numerous papers from all over the world. Particularly the papers by Ewa Rajpert- De Meyts have provided basic molecular evidence for the fetal hypothesis (Rajpert-De Meyts, 2006).

This remarkable story, beginning in 1972, also became the basis of several important original clinical applications, including the early detection of testicular cancer by testicular biopsy in at risk groups (Berthelsen *et al.*, 1982), and prevention of orchiectomy of the contralateral testis for the second neoplasia in that testis by localized irradiation if CIS was present (von deer Maase *et al.*, 1986). The CIS work also changed chapters in textbooks on pathogenesis of testicular cancer (Skakkebaek and Berthelsen, 1981). Earlier it had been assumed that seminomas developed from intratubular germ cells, whereas other germ cell tumours had a different origin in blastomeres displaced in early embryonic life.

Skakkebaek has had the advantage that he is both a clinical endocrinologist and a basic researcher working with testicular specimens. The London based pathologist Connie Parkinson and her urological colleague Harland write in a commentary in *Histopathology* in 2002 (Skakkebaek, 2002): "The fact that, as a clinical endocrinologist, he (Skakkebaek) had the patient's complete histories may make histopathologists envious but in no way diminishes the clinic-pathological importance of his description and evidence-based proof of CIS of the testis"

The early findings from the 1970s have been followed up by numerous projects in the Copenhagen laboratory about CIS testis and testicular cancer, basic as well as clinical (Fig. 3). Jørn Müller worked on testicular samples from children and men with disorders of sex development (DSD) and characterized the ploidy in these cells. Aneuploidy was a frequent feature of the cells, further emphasizing the neoplastic nature of CIS (Müller and Skakkebaek, 1981). He also showed that children with androgen insensitivity syndrome had an increased risk to harbour CIS in their gonads

(Müller and Skakkebaek, 1984; Müller *et al.*, 1985). The same was true for those with gonadal dysgenesis and the 45,XO/46,XY karyotype. Søren Krabbe and Aleksander Giwercman showed that men with a history of cryptorchidism also had an increased risk to have CIS (Krabbe *et al.*, 1979; Giwercman *et al.*, 1987). All this fitted well to the epidemiological findings according to which these groups had an increased risk to develop testicular cancer. These early papers together with the basic research programme developed by Ewa Rajpert-De Meyts (See her article pp) led up to the current hypothesis of testicular dysgenesis syndrome (TDS) (Skakkebaek *et al.*, 2001), by which testicular germ cell cancer is associated with impaired



Fig. 2. During the past 33 years the Copenhagen group has organized 7 international workshops on carcinoma *in situ* and cancer of the testis. The first was held in 1980 and the latest in 2010. The proceedings were published in various journals, including *International Journal of Andrology*, *European Urology* and *APMIS*. Dr. Kenneth M Grigor participated in all of them as the editor.



Fig. 3. Department of Growth and Reproduction at its early days in the 1990s when everybody looked still so young. Niels E. Skakkebaek stands in the centre with Jørn Müller on his right-hand side and Ewa Rajpert-De Meyts between them (behind).

spermatogenesis, cryptorchidism and hypospadias, including some cases of DSD. According to the TDS hypothesis these disorders may have a common origin in the developing testis.

Thus a clinical science story on the origin of testicular germ cell cancer that started with a couple of infertile patients in 1972 has not yet come to the end. On the contrary, the Copenhagen CIS and testis cancer group is more active than ever, now with Ewa Rajpert-De Meyts as the chief for an extensive programme on germ cell cancer. The group is carrying out forefront translational research with a focus on the fetal hypothesis and possible roles of genetic and environmental factors in testicular germ cell cancer. In addition, a member of the group, Kristian Almstrup is leading a team attempting to diagnose CIS cells in semen of men with CIS. Interestingly, the group has provided good evidence that CIS cells in some patients are exfoliated into seminal fluid. Rajpert-De Meyts and Almstrup found that this method is particularly relevant to use in screening of infertile men for germ cell neoplasia, the same population of patients from which Skakkebaek's first CIS patients were derived.

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