Atlas of Human Experimental Teratomas

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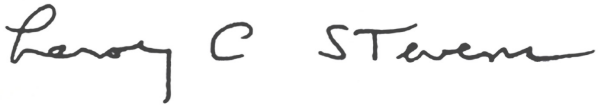
Leroy C. Stevens (1920-2015)



This Atlas of Teratomas derived from xenografted human pluripotent embryonic stem cells is dedicated to Leroy C. Stevens (1922 -2015), a pioneer of mouse teratoma research and the forefather of all of us working in this field.

Preface

Pluripotent human stem cells: standing on the shoulders of giants

The advent of human pluripotent stem cells, with the first derivation of human embryonic stem cells in 1998 (Thomson *et al.,* 1998), and of human induced pluripotent stem cells in 2007 (Takahashi *et al.,* 2007; Yu *et al.,* 2007), has ushered in an era of considerable excitement about the prospects of using these cells to develop new opportunities for healthcare, from their potential for regenerative medicine to their use as tools for studying the cellular basis of many diseases and the discovery of new drugs. But as with the flowering of many new areas in science, the biology of human pluripotent stem cells has its roots in a long history of, sometimes, less fêted research (Solter, 2006). In a period when research funding is frequently driven by a desire to meet specific clinical or economic goals, it is salutary to remember that the opportunities offered by human pluripotent stem cells have their origins in curiosity-driven research without any of those goals in mind.

In this case, that research focused on the relatively rare gonadal cancers known as teratomas, tumors that have fascinated people since antiquity because of their sometimes grotesque manifestations with haphazard collections of tissues and sometimes recognizable body parts (Damjanov and Solter, 1974). Although well-known to clinical pathologists, it was the pioneering work of Leroy C. Stevens, who first discovered that teratomas occur at a significant rate in the 129 strain of the laboratory mouse and could be produced experimentally (Stevens and Little, 1954; Stevens, 1967), that laid the foundations for our understanding of the biology of these tumors and the central role of the embryonal carcinoma cell, one of the archetypal tumor stem cells.

Teratocarcinomas are teratomas that also contain embryonal carcinoma cells, undifferentiated cells that had been suggested to give rise to the array of somatic cells characterizing these tumors (Friedman and Moore, 1946). In a groundbreaking study, Kleinsmith and Pierce (1964) showed that single cells from a teratocarcinoma of the laboratory mouse could generate a new teratocarcinoma after transplantation to a new host. These results, in parallel with studies in the hematopoietic system (Till and McCullough, 1961) and other adult tissues, such as the skin and intestine (Potten and Lajtha, 1982) led to the development of the concepts of stem cells with their dual properties of indefinite proliferation (self-renewal) and a capacity to initiate differentiation into terminal, functional cell types. From these observations, Pierce developed the notion that tumors in general represent caricatures of their tissue of origin, and arise from defects in differentiation, as much as from dysregulated proliferation (Pierce 1974).

Stevens, and also Nikola Skreb and his colleagues, confirmed suspicions of a close link between teratomas and embryos by showing that these tumors could also be derived from embryos transplanted to ectopic sites (Stevens, 1970; Solter *et al.,* 1970). Meanwhile, lines of embryonal carcinoma cells were established *in vitro* (Finch and Ephrussi, 1967). These cultured cells remained pluripotent, being able to differentiate, not only into teratomas *in vivo*, but also when grown in culture (Jakob *et al.,* 1973; Martin and Evans, 1974). The dramatic finding of Brinster (1974) that these cultured tumor stem cells were also able to contribute to normal embryonic development after transfer to a blastocyst to yield a chimeric mouse, confirmed by Papaioannou *et al.* (1975) and Mintz and Illmensee (1975), provided a final confirmation that embryonal carcinoma cells were caricatures of the pluripotent inner cell mass cells of the early embryo. In a final turn of the wheel, Evans and Kaufman (1981) and, separately, Martin (1981), showed that murine pluripotent cells could be derived directly from the inner cell mass cultured *in vitro* under conditions that had been developed for the maintenance of embryonal carcinoma cells. These embryonic stem cells differed from the embryonal carcinomas cells by being more efficient at forming embryo chimeras, which included germ line chimerism, probably because they had not adapted genetically to tumor growth. But they too would form teratomas when grown in adult mice.

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As with murine embryonic stem cells, the development of human embryonic stem cells was preceded by studies of human embryonal carcinoma cells derived from teratocarcinomas, obtained during therapy, beginning with xenografted lines established by Pierce in the 1950's (Pierce *et al.,* 1957; Fogh and Trempe, 1975; Hogan *et al.,* 1976; Andrews *et al.,* 1980). Several of these lines were able to differentiate extensively both *in vitro* and *in vivo*, in xenograft tumors grown in immunosuppressed mice (Andrews *et al.,* 1984; Andrews, 1984; Pera *et al.,* 1989; Damjanov *et al.,* 1993). Nevertheless, human embryonic stem cells, when derived directly from human embryos, proved to have a much wider capacity to differentiate, and to be karyotypically diploid, opening up the prospects for their use in regenerative medicine (Thomson *et al.,* 1998; Reubinoff *et al.,* 2000).

With thoughts of developing applications, the need for tools for characterizing these cells became paramount. To this end, the International Stem Cell Initiative (ISCI) was founded in 2004 as a consortium of researchers from around the world, funded by the International Stem Cell Forum (http://www.stem-cell-forum.net/), focused on developing standards for human pluripotent stem cell biology. Among studies carried out by the ISCI were the characterization of markers of the undifferentiated cells (ISCI, 2007), a comparison of culture media (ISCI, 2010) and a study of the common genetic variants that occur in human pluripotent stem cells on prolonged culture *in vitro* (ISCI, 2011). Nevertheless, central to work with both embryonic stem cells and, perhaps, even more so with induced pluripotent stem cells, is the ability to confirm their pluripotency. For human cells, the gold standard has been their ability to form teratomas containing derivatives of all three embryonic germ layers, ectoderm, mesoderm and endoderm, following growth in immunocompromised mice. Although other, *in vitro*, tests of differentiation capacity are also used, teratomas provide not only insights into differentiation capacity itself, but also the ability of the cells to form organoid structures that can aid the recognition of particular cell lineages. Importantly, teratomas also offer insights into the potential malignancy of the undifferentiated cells and some of their derivatives, building on a long experience of the clinical pathology of these tumors. In this Atlas, produced on behalf of the ISCI, we have harked back to the clinical pathology of human teratomas to provide a guide for those reviewing the histology of xenograft tumors produced by human pluripotent stem cells.

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