

# Tumor Stem Cells

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## MILESTONES

Kleinsmith LJ, Pierce GB:

**Multipotentiality of single embryonal carcinoma cells.**  
*Cancer Research* 1964 24:1544-1551

Pierce GB, Wallace C:

**Differentiation of malignant to benign cells.**  
*Cancer Research* 1971, 131:127-134



Gordon B. Pierce

In ground-breaking research published in 1964, Barry Pierce, then a faculty member in the Department of Pathology at the University of Michigan, and L.J. Kleinsmith, a student fellow in his laboratory, demonstrated that single undifferentiated cells isolated from a murine teratocarcinoma, when transferred into normal mice, gave rise to

malignant teratocarcinomas that contained differentiated tissues representative of all three major germ cell layers<sup>1</sup>. When examined by light microscopy, the teratocarcinomas consisted of foci of undifferentiated malignant cells interspersed with disorganized arrays of adult somatic tissues representative of endoderm, mesoderm and ectoderm in various stages of differentiation. The differentiated tissues included neural, gastrointestinal, skin, muscle, bone, cartilage, marrow, notochord and yolk sac. By morphological criteria, the differentiated somatic tissues in the teratocarcinomas were considered to be non-malignant. Pierce concluded that these murine teratocarcinomas were malignant tumors that consisted of a pool of replicating, multipotential tumor stem cells that gave rise to nonreplicating differentiated cells whose organization mimicked normal tissue development. Based on these findings and on histological features routinely observed in many human and experimental cancers, Pierce hypothesized that most cancers contained a pool of malignant stem cells, some of whose progeny differentiated into nonmalignant, post-mitotic tumor cells. In effect, Pierce was proposing that a malignant cell could become benign. This concept challenged the dogma "once a cancer cell, always a cancer cell," and Pierce found little enthusiasm for his concept amongst cancer researchers. At the time the studies were published it was generally believed by oncologists that teratocarcinomas were not representative of other cancers and, as interesting as these tumors might be, they were oddities and not relevant to cancer in general. There were several unique characteristics of murine teratocarcinomas that

fostered this skepticism. Spontaneous testicular teratocarcinomas occurred only in the 129 strain of mice, these tumors developed in the gonad during fetal life, and it was possible to experimentally induce teratocarcinomas by injecting normal primordial stem cells from blastocysts of 129 strain mice into the testes of adult mice. Perhaps also operating in the background was the longstanding speculation by some scholars that teratocarcinomas actually reflected aberrant parthenogenetic embryogenesis, a concept that is likely related to their designation by some as *embryomas*.

Pierce's concept linking differentiation and cancer was supported by the startling finding of Leroy Stevens<sup>2</sup> that the normal pluripotent embryonic stem cells in a murine blastocyst, cells which if left in the blastocyst would develop into a mouse, developed into a teratoma or teratocarcinoma if they were injected into an adult testis. This finding appeared to complete a transformation circuit that linked tumorigenesis, embryogenesis and differentiation because, as already mentioned, Pierce had shown that cancer cells could give rise to normal differentiated adult cells. As a diagnostic pathologist, Pierce was aware of rare clinical instances in which highly malignant cancer cells in a patient appeared to spontaneously differentiate and the tumor regressed.

When such cases were reported in the literature, they were considered medical curiosities for which there was no explanation. An example is the report in *The American Journal of Pathology* in 1927 by Harvey Cushing and S.B. Wolbach that described a patient with a neuroblastoma in which the malignant neuroblasts spontaneously differentiated into mature ganglion cells to form a benign ganglioneuroma. Convinced of the merit of his concept, and having moved to the Department of Pathology at the University of Colorado, Pierce expanded the scope of his research to include investigations of other cancers besides the murine teratocarcinomas.

In a milestone paper published in *Cancer Research* in 1971<sup>3</sup>, Pierce and Wallace used a

rat squamous cell carcinoma to test the hypothesis that the cancer contained a pool of proliferating stem cells and a pool of non-proliferating, post-mitotic differentiated cells. Microscopically this cancer consisted of foci of heavily keratinized flattened epithelial cells designated as "keratin pearls" that were reminiscent of the cells in the upper layer of normal skin. These foci were separated from each other by areas of undifferentiated cancer cells, many of which contained mitotic figures. When rats bearing this cancer were injected with tritiated thymidine and the tumors examined at various time intervals afterwards using light and electron microscopic autoradiography, it was observed that two hours after injection the thymidine label was present almost exclusively in the undifferentiated cells of the tumor. During the 96-hour period of observation there was a progressive increase in the number of labeled cells that were present in the highly differentiated areas of the tumor.

The investigators concluded that the cells in the keratin pearls were not synthesizing DNA and that the growth of the pearls depended on incorporation of undifferentiated cancer cells into the pearls with subsequent differentiation. The electron microscopic analyses expanded and confirmed these findings. The initial thymidine incorporation occurred in ultrastructurally undifferentiated cancer cells and later the label appeared in tumor cells that had desmosomes and other features of the cells of the normal stratum spinosum of skin. At even later times the label appeared in tumor cells that had features of granular layer cells of normal skin. In addition to these morphological findings, Pierce and Wallace microdissected undifferentiated areas and differentiated areas from the cancer and transplanted these into normal rats. Squamous cell carcinomas developed in about a third of the rats injected with undifferentiated cells, but in none of the rats injected with differentiated cells. In later studies, Pierce and colleagues investigated chondrosarcomas, and adenocarcinomas of the breast and colon and made comparable findings and conclusions.

In addition to the fundamental knowledge that these studies contributed to understanding the role of differentiation in cancer, they established the foundation of a novel strategy for treating cancers based on inducing the differentiation of malignant cells to a post-mitotic state. The investigations of Barry Pierce and Leroy Stevens in the murine teratocarcinoma model facilitated the discoveries by Brinster<sup>4</sup> and by Illmensee and Mintz<sup>5</sup> that the malignant stem cells of the 129 strain teratocarcinomas, when injected into normal blastocysts from other strains of mice, produced normal offspring that were genetic mosaics. Thus, the same tumor stem cells that produced teratocarcinomas when injected into adult testes were found to differentiate into the full range of normal adult tissues in the mosaic mice that were produced when injected into normal blastocysts. These findings eventually led to the development of 129 strain teratocarcinoma stem cells as tools for constructing transgenic<sup>6</sup> and gene knockout mice. While considered by many in the beginning as non-relevant oddities, teratocarcinomas have yielded an abundance of fundamental knowledge about developmental biology and the pathobiology of cancer, and have contributed to the development of some of the most powerful genetic tools currently in use.

#### References

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