Homeobox genes and cancer: New OCTaves for an old tune

In this issue of *Cancer Cell*, Gidekel et al. demonstrate that Oct-4, a member of the POU class of homeobox genes, is a critical player in the genesis of testicular germ cell tumors. This study provides further evidence that deregulated expression of homeobox genes, which occurs in many solid tumors, is functionally relevant for carcinogenesis and highlights unique features that distinguish homeobox genes from other cancer-promoting genes.

The intimate relationship between embryogenesis and oncogenesis has long been a prevailing theme in cancer biology. This theme is well exemplified by the homeobox gene family, whose various members mediate a plethora of embryonic functions, while their deregulation may be associated with tumorigenesis. Nonetheless, despite the many instances in which aberrant homeobox gene expression has been found to occur in solid tumors, until recently there have been relatively few cases in which such misexpression has been definitively associated with carcinogenesis (Abate-Shen, 2002). The study by Gidekel et al. (2003) provides an elegant example, showing that a homeobox gene that is normally required for differentiation of a particular tissue can play a causal role in carcinogenesis of that same tissue if expressed at the wrong time, at the wrong levels, or in the wrong contexts. In particular, the authors demonstrate that *Oct-4* (also known as *Oct-3* and *POU5f1*), which is normally expressed in germ cells and is required for their maintaining their pluripotency (Nichols et al., 1998; Niwa et al., 2000), can promote tumorigenesis when expressed inappropriately in these same cells.

In normal circumstances, *Oct-4* is restricted to pluripotent cells of the early embryo and those of the germ cell lineage, while its downregulation is associated with loss of pluripotentiality of these cells (Nichols et al., 1998; Niwa et al., 2000). The study by Gidekel et al., as well as work of Looijenga and colleagues (Looijenga et al., 2003), show that *Oct-4* is expressed in each of the various types of testicular germ cell tumors, which comprise a heterogeneous group of neoplasia, including embryonal carcinomas, seminomas, and mixed germ cell tumors, as well as their common precursor, intratubular germ cell neoplasia. Although *Oct-4* expression is observed in most, if not all, testicular germ cell tumors, it is not found in other solid tumors (Gidekel et al., 2003; Looijenga et al., 2003).

During development, *Oct-4* expression is required to maintain the pluripotency of the inner cell mass (ICM) cells, which give rise to all fetal cell types; in the absence of *Oct-4*, ICM cells differentiate into trophoectoderm, which gives rise to extraembryonic tissue (Nichols et al., 1998; Niwa et al., 2000). Using a cleverly engineered series of embryonal stem (ES) cells in which the levels of *Oct-4* expression can be controlled from 0% to 150% of wild-type by varying the number of *Oct-4* alleles and by using an inducible exogenous transgene (Niwa et al., 2000), Austin Smith and colleagues showed that the amount of *Oct-4* can determine cell fate (Figure 1). Thus, the lowest levels of *Oct-4* expression lead to trophoectoderm differentiation, intermediate levels are associated with the pluripotent stem cells, and the highest levels promote differentiation of primitive endoderm (Niwa et al., 2000).

These same ES cells have now been used to demonstrate that the dosage of Oct-4 protein is directly related to the tumorigenic potential of these cells (Figure 1). In particular, Gidekel et al. find that as *Oct-4* expression is increased from 0% to 150% of wild-type, the potential for these ES cells to form tumors in syngeneic hosts shifts from about 4% at the lowest level of *Oct-4* expression to better than 80% at the highest levels and is associated with an increased accumulation of malignant versus nonmalignant cells (Gidekel et al., 2003).

Not only is tumor incidence coincident with the levels of *Oct-4* expression, their sustained growth is dependent on its continued expression since the tumors regressed upon lowering *Oct-4* expression (i.e., by turning off the inducible exogenous gene). This is analogous to the tumor dependence of other oncogenes such as RAS, for example, in which its removal by turning off the inducible gene also leads to tumor regression (Chin, 2003), whereas elimination of other oncogenes, such as *Myc*, does not lead to tumor regression under all circumstances (Pelengaris et al., 2002).

The implication of these findings is that some, but not all, tumors may require a single oncogene for maintenance of the transformed state. If these observations are confirmed to be relevant for the growth of testicular germ cell tumors in humans, *Oct-4* would represent an attractive target for therapeutic intervention. Importantly, because the level of Oct-4 expression is critical for

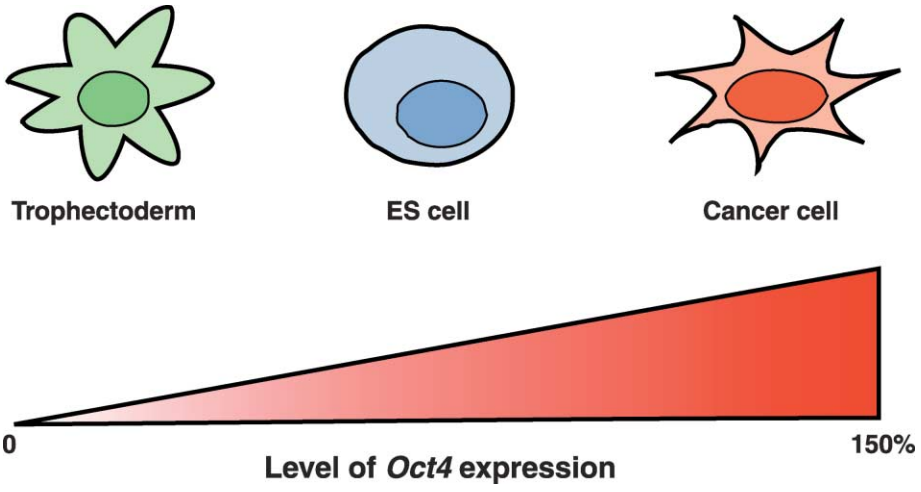

Figure 1. Diagram showing the relationship of *Oct-4* expression levels to the occurrence of normal and cancer cell types

tumorigenesis, it would only be necessary to lower its expression to achieve the desired outcome, which is of course more feasible than eliminating it totally.

In a broader context, the study by Gidekel et al., as well as previous studies in the literature (Abate-Shen, 2002), contribute to our understanding of how homeobox genes may be functionally relevant for oncogenesis, as well as how they may differ from other classes of cancer-promoting genes. First of all, deregulated expression of homeobox genes in solid tumors typically displays tissue specificity; although *Oct-4* is expressed in virtually all germ cell tumors, it is not expressed in other solid tumors. In this regard, homeobox genes provide attractive candidates for modulating the tissue-specific features of cancer phenotypes, presumably acting in conjunction with broad-spectrum oncogenes or tumor suppressors.

Secondly, deregulation of homeobox genes may occur early in carcinogenesis; expression of *Oct-4* was prevalent in precursor lesions of germ cell tumors as well as in the tumors themselves. This parallels the situation with other homeobox genes, including *Nkx3.1* and *Cdx2*, which are also downregulated at the earliest stages of carcinogenesis of the prostate and colon, respectively. Considering that homeobox genes are likely to regulate the differentiation status of tissues in which they are expressed, one can speculate that their deregulated expression perturbs the normal differentiation program, thereby predisposing to neoplasia.

Thirdly, homeobox genes are unusual in the sense that their deregulation can be manifest either as an up- or downregulation of expression, depending upon the particular gene and the timing of its expression in normal scenarios (Abate-Shen, 2002). Thus, *Oct-4* falls into the category of homeobox genes whose expression during development is restricted to undifferentiated precursors of developing tissues, while its expression is reactivated in neoplasia of these tissues. This contrasts with the category of homeobox genes that are normally expressed in differentiated adult tissues, but then downregulated in cancer, examples of which include *Nkx3.1* in the prostate and *Cdx2* in the colon.

Finally, the functions of homeobox genes appear to be exquisitely sensitive to gene dosage, which is well illustrated in the study by Gidekel et al. One possibility is that the sensitivity to dosage reflects the significance of protein-protein interactions for mediating homeoprotein functions; in other words, a 2-fold difference in levels of a homeoprotein could translate into profound effects on gene expression simply by perturbing the balance of protein interactions. Alternatively, slight variations in homeobox gene expression may influence the expression or functions of other homeobox genes that may also be dosage dependent. For example, the newly discovered homeobox gene, *Nanog*, plays a role in maintaining the pluripotency of embryonic stem cells and germ cells and, like *Oct-4*, its functions are sensitive to variations in dosage (Chambers et al., 2003; Mitsui et al., 2003). It would be of interest to see if variations in *Oct-4* expression levels affect the expression and/or function of *Nanog*, either in normal germ cells or in germ cell tumors.

In the past, the functional significance of deregulated homeobox gene expression in solid tumors has been suspect, in part because of their relatively subtle effects and because they display relatively modest differences in expression levels in cancerous versus noncancerous cells. The study by Gidekel et al. lends new credence to the idea that deregulation of homeobox genes can indeed be important in the oncogenic process, perhaps by affecting the differentiation status of the tissues in which they are expressed. Furthermore, their tissue specificity, association with early stages of carcinogenesis, and sensitivity to dosage make homeobox genes excellent candidates for prognostic indicators, as well as targets for early intervention.

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Selected reading

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