CHAPTER 2

pRb in the Differentiation of Normal and Neoplastic Cells

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Abstract

This chapter deals with the role played by the retinoblastoma protein (pRb) in a variety of differentiation processes. After broadly reviewing the current knowledge on this issue, it points at two common themes. The first is the exclusive involvement of pRb in the final maturation stages of each lineage, so that the functional ablation of the protein produces relatively subtle differentiation defects. The second is that, at least in the cell types more thoroughly investigated, pRb exerts its pro-differentiation potential by enhancing the activities of transcription factors that are key regulators of tissue-specific differentiation.

Finally, the hypothesis is put forward that pRb plays a role in the final differentiation stages of a much wider range of cell types than currently recognized. It is proposed that one reason for the well-know, poorly-understood, inverse relationship between differentiation and malignancy is the functional impairment of pRb and possibly its family members in the vast majority of human cancers.

Introduction

Tumor cells are uniformly characterized by unchecked proliferation on one side and impaired or arrested differentiation on the other. In general, the degree of differentiation of tumors correlates inversely with their malignancy, the more undifferentiated neoplasias being also the more aggressive. The mechanisms through which altered proliferation and impaired differentiation are linked are only partially understood.

The general rule that all malignant tumors show altered differentiation applies even to malignancies ostensibly comprised of highly or even terminally differentiated cells. A case in point is that of multiple myeloma. In this disease, the vast majority of tumor cells are terminally differentiated plasma cells, while the malignancy grows through the expansion of a minor compartment of cells whose differentiation is arrested at a stage compatible with proliferation. Conversely differentiation, when allowed to proceed in a tumor cell, can take over cell cycle control and bring the neoplastic cell to a halt. It is well known that differentiation tends to oppose cellular transformation. In extreme but not uncommon cases, terminal differentiation coexists with malignant transformation. Although terminally differentiated cells are unable to proliferate by definition and cannot possibly be transformed, spontaneous terminal differentiation often takes place even in highly aggressive cancers in a fraction—sometimes the majority—of the tumor cells. Such cells cease proliferating, thus demonstrating that differentiation can suppress the transforming events that lead to tumorigenesis. By way of example, in the

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solid tumor rhabdomyosarcoma, most cells are often undifferentiated and morphologically uncharacteristic. However, a variable percentage of cells derived from the malignant clone terminally differentiate into muscle fibers. Understanding the molecular underpinnings of the conflict between neoplastic transformation and differentiation is a fundamental question in cancer biology. As it is true of all basic questions in cancer, finding answers should provide us with potential targets for therapeutic interventions. Indeed, it should be stressed that differentiation of some tumors can also be elicited both in vitro and in vivo by chemical treatments. We must understand the molecular mechanisms through which differentiation can force a tumor cell to stop as in the instances of differentiation therapies for myeloid leukemias and neuroblastomas.

The universal character of the transformation/differentiation antithesis suggests that one or very few mechanisms underlay it. While it is known that a large number of genetic and epigenetic alterations concur to cell transformation in a seemingly endless variety of combinations, we suggest that most or all transforming mechanisms share a common theme entailing impaired differentiation. Specifically, we hypothesize that one fundamental reason for the inverse correlation between differentiation and malignancy is the near-universal inactivation of the retinoblastoma protein (pRb) pathway in human tumors. Such inactivation alters the control of the cell cycle and contributes to determine the unchecked proliferation of tumor cells. At the same time, we contend, it impairs cell differentiation in a much wider variety of cell types than currently appreciated. Impaired differentiation is far from being a marginal byproduct of Rb pathway inactivation. On the contrary, it is a necessary condition for sustained proliferation of tumor cells in those cases in which their normal counterparts terminally differentiate into nonproliferating or postmitotic cells. This is very frequently true, as for example in the cases of most epithelial and hematopoietic cells which are almost always postmitotic in the final stages of their differentiation. Even when the differentiation of a given cell type is nonterminal (e.g., hepatocytes, thyrocytes), it is still accompanied by very low proliferation rates which are hardly compatible with neoplastic transformation.

The pRb Pathway in Normal and Neoplastic Cells

The tumor suppressor protein pRb is a central regulator of cell homeostasis, involved in the control of such critical functions as proliferation, differentiation, and programmed cell death. In the cell cycle, pRb exerts its activity in close proximity to the restriction point, regulating the decision to enter S phase. In its cell cycle regulatory capacity, pRb is primarily regulated through phosphorylation. Un- or hypo-phosphorylated pRb is conventionally regarded as "active" and prevents entry into S phase. During G1 phase of an unperturbed cell cycle, pRb is progressively phosphorylated by the cyclin D-dependent kinases cdk4 and cdk6 and the cyclin E/cyclin A-dependent cdk2. Phosphorylation of pRb "inactivates" it, thereby allowing advancement into S phase. pRb phosphorylation allows cell cycle progression mainly by releasing transcription factors of the E2F family. The E2F factors, when bound by hypophosphorylated pRb, form complexes that bind target promoters bearing E2F binding sites and actively repress transcription. Upon phosphorylation, pRb releases the E2F factors, that promote transcription of a large number of genes, many of which are essential regulators or direct effectors of DNA replication. The kinases that phosphorylate pRb are controlled by a variety of mechanisms at different levels. One prominent regulation is exerted by two groups of inhibitory molecules, the INK4 and KIP families. The INK4 family consists of four members, commonly indicated as p15, p16, p18, and p19 from their molecular weights. The INK4 inhibitors have binding specificity for the cyclin D-dependent cdk4 and cdk6 kinases and, when bound to them, prevent their forming complexes with the activating cyclins. The KIP inhibitors include p21, p27, and p57, have the ability to bind all cyclin/ckd complexes or their cyclin moieties alone, either way inhibiting cdk activity.

This highly simplified view of cell cycle regulation in G1 is summarized in Figure 1. It includes only those players whose alterations are frequently involved in pRb pathway inactivation in the course of neoplastic transformation. It is designed solely to serve this discussion,