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Disruption of Cell Cycle Machinery in Pancreatic Cancer

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1. Introduction

This chapter discusses the role of cell cycle machinery in initiation and progression of pancreatic cancer. Normal pancreatic cells—their types, organization, and functions—are first described to characterize the environment in which cellular transformation and tumor expansion occurs. The epidemiology and histology of pancreatic cancer is then briefly presented to emphasize the urgent need for earlier diagnosis and more effective treatments. Current efforts towards this goal are focused on understanding the disease at the molecular level, so the hallmarks of cancerous cells are discussed with respect to the progression model of pancreatic cancer development. Because the pancreas is composed of various cell types with different genetic backgrounds and regulatory systems, identifying the cell in which cancer originates is of utmost importance. Molecular mechanisms of normal proliferative control are then presented so that mechanisms by which they are disrupted can be appreciated. Particular attention is paid to how signaling transduction pathways and the cell cycle machinery cooperate to make cell fate decisions at the Restriction point. This analysis sets the stage for evaluating the role of cell cycle control mechanisms in transformation of the initiating cell in pancreatic cancer. The chapter concludes by arguing that genetic alterations associated with pancreatic cancer indicate disrupted cell cycle control mechanisms play a central role in disease development and progression.

2. Pancreatic tissue organization and cellular function

Evaluating the role of cell cycle machinery in pancreatic cancer requires understanding the architecture and cellular organization of this dual-function gland. The pancreas is an approximately six inch long cylindrical organ in the abdomen, located between the stomach and the spine (Romer & Parsons, 1977).

The endocrine component is composed of clusters of alpha, beta, and PP (pancreatic peptide) cell types that form structures called the islets of Langerhans (Jain & Lammert, 2009). These cells produce metabolic hormones involved in energy metabolism. Major cell types and their organization are summarized in Figure 1.

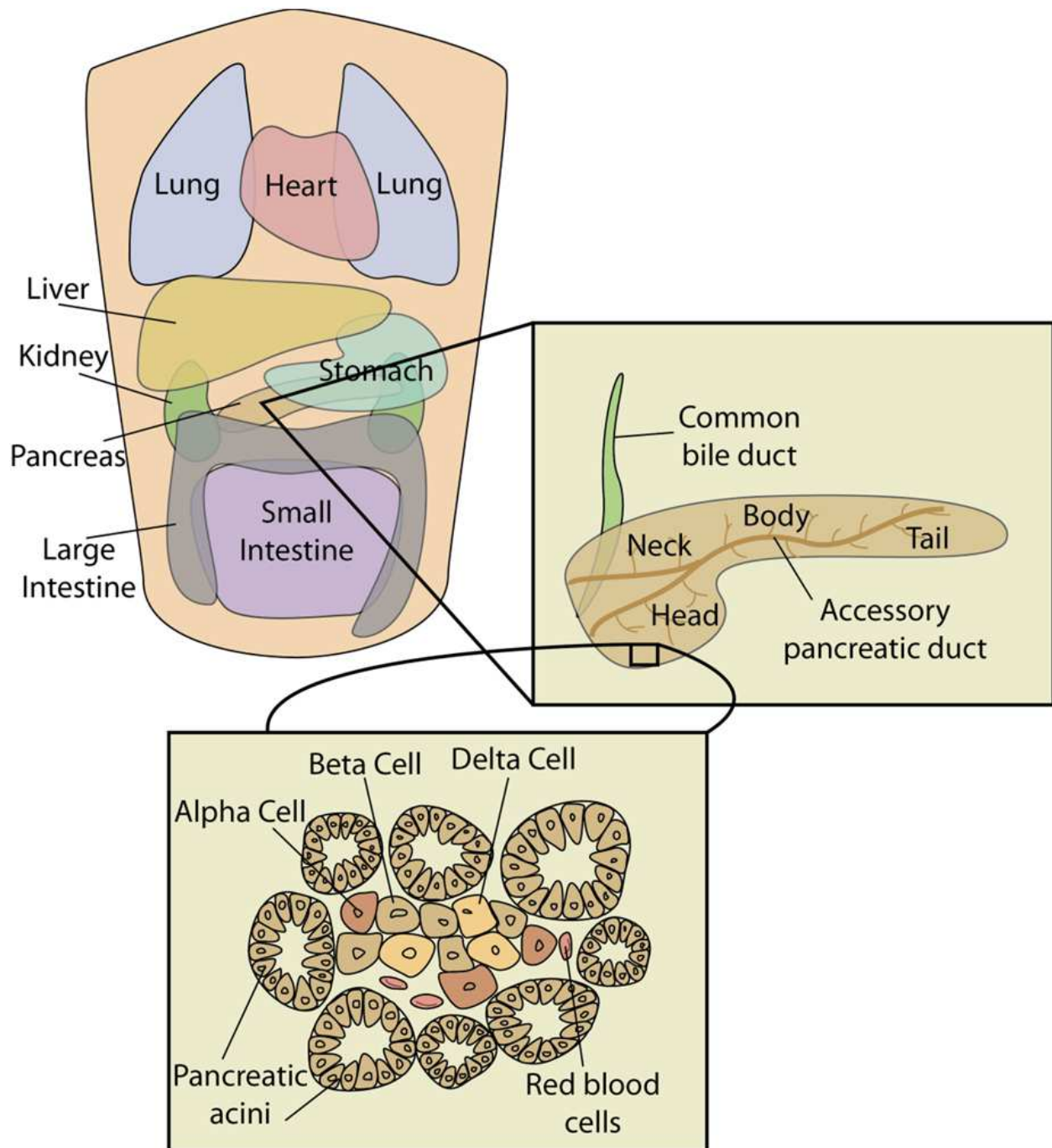


Fig. 1. **Global View of Pancreas.** The pancreas is located in the abdomen behind the stomach. It is composed of four areas: The head, neck, body, and tail. It is comprised of two types of parenchymal tissue: The islets of Langerhans, composed of alpha, beta, and gamma cells are in charge of endocrine signal and hormone detection, while the pancreatic acini are in charge of exocrine signaling and production of digestive enzymes.

It plays major roles in the vertebrate hormonal (endocrine) and digestive (exocrine) systems (Jain & Lammert, 2009, Means & Leach, 2001). The pancreas contains two different types of parenchymal (i.e. functional) tissue that is of endodermal origin (Gittes, 2009). Most of its mass is clustered acinar cells that synthesize digestive pro-enzymes (Means & Leach, 2001).

The most prevalent type of pancreatic cancer is infiltrating ductal adenocarcinoma, which appears to initiate in distinct subsets of cells within the exocrine tissue (Maitra, A & Hruban, 2008). However, other cell types can participate and/or be affected by the disease. This includes “enabling cells”, which are local, untransformed populations that can contribute to disease development. Pancreatic stellate cells, for instance, are stromal cells recruited by the tumor to help create an environment promoting disease progression (Vonlaufen, *et al.*, 2008).

2.1 Endocrine function

Endocrine function is mediated by groups of cells called the islets of Langerhans, which secrete essential peptide hormones regulating energy metabolism into the bloodstream (Jain & Lammert, 2009). The pancreas contains approximately 1 million of these cell clusters, each composed of four different cell types distinguished by their secretory role. The alpha and beta cells work together to maintain blood sugar levels. Alpha cells produce glucagon to promote release of stored glucose in response to an unfed state (Gromada, *et al.*, 2007). In contrast, β cells generate insulin in response to eating so that incoming glucose can be utilized by body tissues (Collombat, *et al.*, 2010). These functionalities are fine-tuned by somatostatin secreted from δ cells (Brink, 2003). PP cells are so called because they produce pancreatic polypeptide that helps regulate endocrine and exocrine secretions, control hepatic glycogen levels, and participate in regulation of gastrointestinal secretions (Lonovics, *et al.*, 1981). Insulin and glucagon are rapidly disseminated by a capillary network that is connected to blood vessels via layers of endocrine cells (Jain & Lammert, 2009, Means & Leach, 2001). Less than 10% of pancreatic cancers originate in endocrine cells. Nevertheless, these cells could play an important secondary role in more common ductal adenocarcinomas via their ability to produce hormones affecting cell fate decisions. In addition, their extensive capillary network could be exploited by metastasizing tumor cells.

2.2 Exocrine function

The majority of pancreatic cancers (>90%) are infiltrating ductal adenocarcinomas of the exocrine system (Maitra, A & Hruban, 2008). Thus, identifying the potential cell types involved and their normal function is essential for evaluating how cell cycle machinery contributes to cancer development. Exocrine function is mediated by clusters of acinar cells (called acinus) that secrete bicarbonate ions and digestive pro-enzymes (Means & Leach, 2001). These products are transported in the pancreatic juice to the duodenum by a ductal system lined with a layer of mucinous columnar epithelial cells. Exocrine function is under control of the hormones gastrin, cholecystokinin and secretin, which are secreted by gastrointestinal cells in response to physical distension and food intake (Jean, 2008).

The alkaline bicarbonate secreted by centroacinar cells regulates pH in the small intestine by neutralizing the acidic chyme arriving from the stomach (Freedman & Scheele, 1994). Centroacinar cells also secrete mucins, a family of high-molecular-weight, heavily glycosylated proteins known primarily for forming biological gels (Nagata, *et al.*, 2007). They are involved in signaling, barrier formation, lubrication, and the immune response via binding and/or blocking pathogens (Hollingsworth & Swanson, 2004). Overexpression of mucin proteins (e.g. MUC1) occurs in many different types of cancers, including pancreatic (Moniaux, *et al.*, 2004). Based on their unique genetic background, centroacinar cells have

been implicated as the potential cell of origin giving rise to ductal adenocarcinomas (Singer & Niebergall-Roth, 2009, Stanger & Dor, 2006).

Digestive pro-enzymes for breaking down fats and protein are secreted into the lumen of the acinus by basophilic cells, so-called because they tend to stain intensely with basic dyes (Singer & Niebergall-Roth, 2009). These cells contain large cytoplasmic secretory granules in which digestive pro-enzymes are maintained in an inactive state, a method of regulatory control that is essential to prevent auto-degradation of the cell and subsequent development of a condition called acute pancreatitis (Waldthaler, *et al.*, 2010). This type of cellular damage increases the risk of pancreatic cancer, an observation that provides insight into identity of the originating cell and how it is transformed (Suda, *et al.*, 2007).

3. Pancreatic cancer

Epidemiology studies have revealed pancreatic cancer runs in some families and is associated with specific genetic mutations (Chakraborty, *et al.*, 2011, Greer, *et al.*, 2009, Lowenfels & Maisonneuve, 2006, Shi, *et al.*, 2009). As is the case for all cancers, pancreatic cancer arises when a normal cell is transformed by accumulated genetic alterations into a cancer cell that seeks to duplicate itself at the expense of the organism. If this transformed cell can escape internal and external fail-safe mechanisms, obtain nutrients, and successfully activate its proliferative program, it can eventually form a more complex mass of cancerous cells. Continued growth of the tumor depends on space availability and promoting growth of blood vessels (angiogenesis) to supply nutrients and remove waste. Further tumor growth can result in loss of pancreatic function, impairment of local blood vessels and organs, and finally metastasis and secondary tumor formation at distal parts of the body. An unfortunate characteristic of pancreatic cancer is its propensity for metastasis early in tumor growth, which is likely responsible in part for its aggressive nature (Bardeesy & DePinho, 2002).

Pancreatic cancer is not exceptionally rare, with approximately 43,000 cases in 2010, making it the tenth-most-common form of the disease (ACS, 2007, ACS, 2010, NCI, 2010). However, 37,000 of those died, illustrating its high mortality rate and ranking pancreatic cancer as the fourth leading cause of cancer fatalities worldwide (ACS, 2007, ACS, 2010, NCI, 2010). The lack of early symptoms results in delayed diagnosis and a higher likelihood of metastasis, thus dramatically limiting treatment options and outcome (Hansel, *et al.*, 2003). The percent of patients alive one year after diagnosis is about 25%, while the five year rate is a depressing 6% (Hariharan, *et al.*, 2008). The five year survival increases substantially with early detection and surgical removal of the tumor (approximately 22%), emphasizing the importance of developing better diagnostic tools and markers (Benson, *et al.*, 2010). Even with early detection and conventional treatment, however, pancreatic cancer is still quite deadly. The ineffectiveness of standard therapy suggests its development and aggressiveness might involve unique molecular features.

The pancreas is composed of head, body, and tail sections, with cancer most commonly occurring in the head region (see Figure 1) (Romer & Parsons, 1977). The majority of cases are malignant adenocarcinomas arising in the exocrine component (Maitra, A & Hruban, 2008). Typically, the cancer initiates in the pancreatic ducts (infiltrating ductal adenocarcinoma), or less frequently in the acini (acinar adenocarcinoma). It presents as a

dense mass with crennolated extensions into surrounding tissue. Histological analysis reveals a neoplastic epithelium composed of poorly differentiated, gland-forming cells that illicit a very strong growth of fibrous and/or connective tissue around the neoplaisa (i.e. a desmoplastic reaction) (Hartel, *et al.*, 2004, Maitra, Anirban, *et al.*, 2006). A much smaller subset of exocrine pancreatic tumors present as adenosquamous carcinomas, squamous cell carcinomas, and giant cell carcinomas (Hruban & Zamboni, 2009). Metastatic endocrine cancers (also called islet cell tumors) are much less common and only account for approximately 1% of pancreatic cancers (Spiegel & Libutti, 2010).

3.1 Molecular events underlying cellular transformation

Cancers are recognized as such despite diverse physiological presentation because they all share a limited set of underlying causative characteristics. These so-called “hallmarks of cancer” are the distillation of extensive efforts to understand how a normal cell is transformed into a cancerous version (Hanahan & Weinberg, 2000).

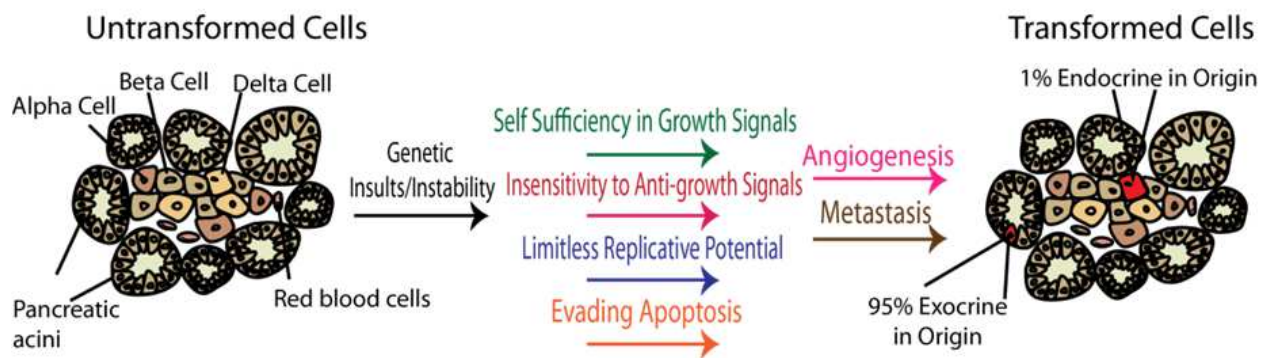


Fig. 2. **Hallmarks of Cancer.** Genetic insults to the pancreatic cells can result in the accumulation of the six hallmarks associated with cancer. The relative percentage of transformed cells types in the pancreas is also shown.

For this reason they are the predominant paradigm for understanding its molecular basis and developing better diagnostics and treatments. In order to evaluate how disrupted cell cycle control specifically contributes to pancreatic cancer, it is first necessary to appreciate why it has been designated as a fundamental hallmark of the disease.

3.2 Hallmarks of cancer

Normal cells perform specific tasks and exist for the greater good of the organism. For this reason, their proliferative capability is a double-edged sword that must be highly constrained by internal checkpoints and external signals from other cells or the microenvironment (Bogenrieder & Herlyn, 2003). Cancer cells evade these constraints via accumulated genetic alterations, resulting in a selfish cell whose allegiance is now to its own survival and expansion. A multi-cellular organism protects itself by making cell duplication dependent on externally generated signals. Positive growth-promoting factors are required for proliferation while negative growth-inhibitory factors must be withdrawn. Two hallmarks of cancer are therefore self-sufficiency in growth signals (i.e. cell division in the absence of mitogenic factors) and insensitivity to growth-inhibitory factors (i.e. cell division

despite the presence of anti-mitogenic signals) (Hanahan & Weinberg, 2000). Extensive analysis of cell signaling pathways has identified myriad ways in which genetic alterations can satisfy these two criteria (Brognard & Hunter, 2011). Typically, multiple biological targets must be compromised due to redundancies and control mechanisms that evolved to prevent deregulated signaling.

Self-sufficiency in growth signals and insensitivity to growth-inhibitory factors is not sufficient to generate a transformed cell capable of tumor formation. Most cells have an internal clock that limits their replicative potential so that they can only duplicate a fixed number of times before entering a senescent or non proliferative state (Hornsby, 2005). As will be discussed in more detail below, stem cells (and cell populations with stem cell-like characteristics) are an important exception to this rule and likely play a central role in the initiation of pancreatic cancer. The mechanism of this clock centers on telomere maintenance, the process whereby chromosome ends are protected from degradation (Yang, Q., 2008). Loss of telomere protection occurs after a fixed number of duplications, sending a signal that causes cells to exit the proliferative cycle and enter a senescent state (Yibin, *et al.*, 2008). Cancer cells typically overcome this internal checkpoint—for instance by hyperactivation of the telomere synthesizing enzyme telomerase—to acquire the proliferative capacity needed for tumor formation (Artandi & DePinho, 2010). Telomere maintenance is also disrupted in pancreatic cancer, but in a complex manner that varies with disease progression (van Heek, *et al.*, 2002). This pattern provides insight into its origins and development.

Cells have additional mechanisms to prevent inappropriate proliferation and mount a protective response should it occur. In the event of irreparable damage or grow-promoting mutations, for example, the cell can initiate an apoptotic pathway resulting in its death and deconstruction (Wyllie, 2010). A major hallmark of cancer is, thus, evasion of apoptosis (Hanahan & Weinberg, 2000).

Once immortalized, the transformed cell can proliferate and begin to generate cancer cells, comprising the bulk of the tumor. However, cellular expansion can only occur up to a certain point as determined by available space and nutrients. A hallmark of cancer is, therefore, formation of new blood vessels (angiogenesis) so nutrients can be obtained and delivered throughout the tumor and so waste products can be removed (Hanahan & Weinberg, 2000). As a result, the tumor can further increase in size and complexity, compromising not only the affected organ itself but also nearby blood vessels and tissues.

The final hallmark of cancer is perhaps most responsible for threatening organism survival. Even with angiogenesis, tumor size will eventually be constrained by physical barriers. As a consequence, the tumor is subjected to selective pressure, driving invasion of surrounding tissue and metastasis to distal locations (Hanahan & Weinberg, 2000). In this process, cancer cells detach from the primary tumor and secrete enzymes (e.g. MMPs) that allow passage through the extracellular matrix (Singh, *et al.*, 2002). They can then migrate and start secondary tumors in surrounding tissue or in distal areas by commuting through the body's highways (lymph and hematopoietic systems). Once a tumor has metastasized, the potential for successful therapeutic intervention is severely reduced, as is the case with pancreatic cancer (Bardeesy & DePinho, 2002).

3.3 Progression model of pancreatic cancer development

The current view of pancreatic cancer development is summarized in a progression model wherein the temporally ordered accumulation of genetic mutations drive transitions through a series of pre-cancerous lesions culminating with infiltrating ductal adenocarcinoma (in the majority of cases) (Koorstra, *et al.*, 2008a). These steps were defined by histology of precancerous lesions at various stages that are collectively described as pancreatic intraepithelial neoplasias (PanINs) (Koorstra, *et al.*, 2008b). They are further subdivided into PanIN-1A, PanIN-1B, and PanIN-2/3 based on distinct histology and genetic background (Koorstra, *et al.*, 2008b, Koorstra, *et al.*, 2008a). PanINs present as microscopic lesions situated in the smaller pancreatic ducts (Maitra, Anirban, *et al.*, 2005). Genetic analysis of these distinct cell populations revealed ordered accumulation of alterations associated with the more advanced adenocarcinoma (Koorstra, *et al.*, 2008a). The types of genetic insults present in pancreatic cancer are quite diverse and include large chromosomal alterations such as breaks, duplications, deletions, fusions, and translocations (Campbell, *et al.*, 2010). There is also CpG island methylation of promoters, telomere disruptions, and microsatellite instability, along with specific mutations in important oncogenes and tumor suppressors (Lin, *et al.*, 2011, López-Casas & López-Fernández, 2010, Welsch, *et al.*, 2007). Precursor lesions display an increasing proliferation rate as they progress towards an adenocarcinoma (Koorstra, *et al.*, 2008b).

From a molecular biology perspective, three pertinent questions are: 1) What type of cell undergoes transformation? 2) What genetic elements are disrupted? 3) What biological activities are compromised and how do they contribute to transformation? Each of these questions will be addressed, with particular emphasis on the role of cell cycle machinery. The first task is to consider the cell type in which pancreatic cancer originates, since its genetic makeup and regulatory systems will dictate how to interpret genetic alterations associated with the disease.

3.4 Identity of the cell initiating tumor formation

Identifying the cell in which cancer originates is essential for understanding the consequences of genetic alterations and their effect on cell cycle control. This initiating cell has not yet been definitively described, but we can speculate it accumulates genetic mutations that, if not resolved (via repair or cell death), culminate in the hallmarks of cancer. The average age of onset for infiltrating ductal adenocarcinoma is approximately 73 years, indicating it develops in the mature organ (Greer, *et al.*, 2009, Lowenfels & Maisonneuve, 2006). One obvious candidate for transformation is the differentiated acinar ductal cell itself, which could be induced to re-enter the cell cycle and proliferate in opposition to internal and environmental cues. Such a transition seems quite daunting, because the mature cell must first de-differentiate to a more primitive state with proliferative capacity via genomic reorganization and altered gene expression. Secondly, this reverted cell must be induced to proliferate inappropriately, which likely requires disruption of different biological pathways. Thirdly, it is likely that fail-safe systems unique to each transition would have to be inactivated. Despite these hurdles, centroacinar cells are a possible candidate because they reside at the junction between acini and ducts and are the only differentiated cells in the developed pancreas with activated signaling pathways associated with proliferation (Miyamoto, *et al.*, 2003). Thus, fewer genetic changes might be required in this background to generate the initiating transformed cell.

An alternative possibility is that cancer arises from transformation of a pancreatic stem cell. This model posits that the originating cell in cancer development is either a stem cell or has stem cell-like characteristics (Stanger & Dor, 2006). Their defining feature is unlimited self-renewal capabilities. Normal stem cells play key roles in development and tissue maintenance by dividing asymmetrically to give one progeny with self-renewing capacity and another that commits to a differentiation pathway (Leeb, *et al.*, 2011). Cancer stem cells are thought to follow the same process, except that their asymmetrical division generates cancer cells comprising the bulk of the tumor (Clevers, 2011, Stanger & Dor, 2006). Transformation of a cell with proliferative capacity seems more likely because it already exhibits one or more of the hallmarks of cancer. Evidence suggests that stem cells only make up 0.1-1% of the tumor cell population and show greater resistance to chemotherapy and radiation compared to their progeny (Bomken, *et al.*, 2010). Consistent with this idea, subpopulations of pancreatic cancer cells have been isolated that can initiate a new tumor when implanted in mice (Reya, *et al.*, 2001). Given that disease arises in the mature organ, a likely candidate for initial transformation would be a pancreatic adult stem cell. Such a cell type has not yet been identified, but their presence in other organs makes a pancreatic version a distinct possibility (Li, *et al.*, 2007). These observations could help explain the well-known unresponsiveness of pancreatic cancer to traditional chemotherapies (Wang, Zhiwei, *et al.*, 2011c).

A third possibility is involvement of what are called facultative progenitor cells located in the acinar environment (Leach, 2005). In the case of pancreatic injury (e.g. acute pancreatitis), differentiated cells can be recruited back into the proliferative cycle to replace old or damaged cells (Raimondi, *et al.*, 2010). Such cells exhibit a more “stem cell-like” phenotype and hence are more susceptible to transformation. There is experimental evidence supporting this idea. Acute pancreatitis is a risk factor for pancreatic cancer, and, in a chemically induced version of this disease, acinar cells de-differentiate to replenish the cell supply (Guerra, *et al.*, 2007, Jensen, *et al.*, 2005). In addition, growth-factor stimulation can cause acinar cells to undergo an abnormal transdifferentiation event to generate what is called an acinar-to-ductal metaplasia (Husain & Thrower, 2009). Taken together, these observations strongly implicate facultative progenitors as the initiating cell in pancreatic cancer. While further efforts to definitively characterize the relevant cell type are required, armed with the above possibilities we can now discuss the basics of proliferative control mechanisms and the important role of the cell cycle machinery.

4. Molecular mechanisms of proliferative control

The cell cycle is an experimentally based, theoretical construct describing the stages through which a cell proceeds to generate a faithful copy of itself (Vermeulen, *et al.*, 2003). These discrete steps must be transversed in sequential fashion with DNA replication in S phase followed by chromosome segregation and division in M phase. Gap 1 and Gap 2 phases separate DNA replication from chromosome segregation. They are also important for increasing cell mass (e.g. protein synthesis) and monitoring S and M phases to ensure accurate transmission of the genetic material. Progression through the cell cycle is controlled by a diverse group of molecular components collectively called the cell cycle machinery (Suryadinata, *et al.*, 2010). Disruption of this regulatory network is a fundamental event required for the expansion of cancer cells (Hanahan & Weinberg, 2000). Understanding how

this machinery functions at the molecular level and is linked to cell fate decisions is therefore crucial to evaluating its role in development and progression of pancreatic cancer.

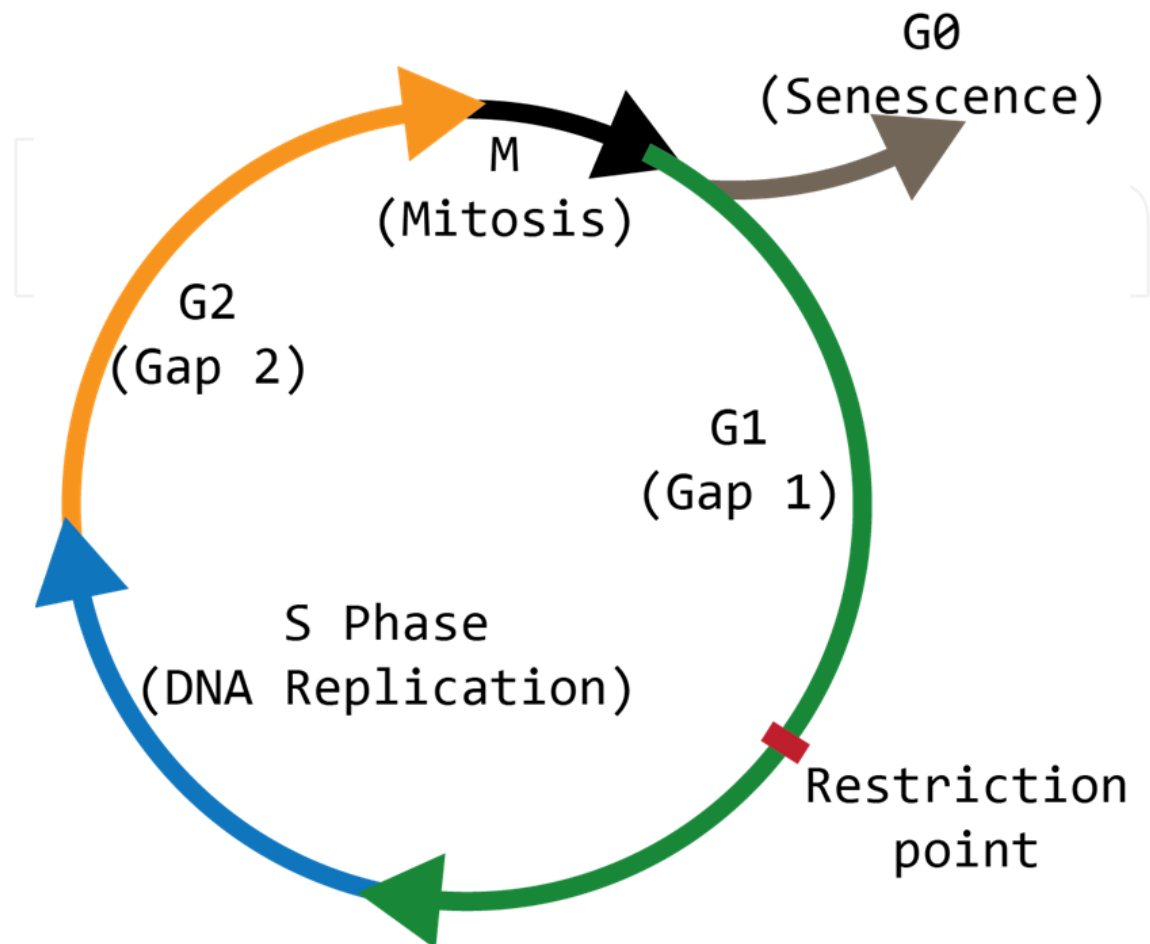


Fig. 3. **The Cell Cycle.** The stages of the cell cycle (G1, S, G2, M) that take place during the duplication of the cell. The Restriction point, the regulatory checkpoint of the cell cycle after which the cell is committed to replication is shown, along with the option of senescence.

4.1 Cell fate decisions

It is essential to evaluate proliferative capacity within the context of cell function and contribution to the organism as a whole. During development, proliferation is transcendent, since exponential cell division is required to generate large number of cells. Once maturity is reached, proliferative capacity is utilized less frequently (e.g. tissue maintenance and repair). A classic example is the hematopoietic system, where immortal stem cells generate progeny destined to become distinct functional cells in the blood (Heike & Nakahata, 2004). As these cells proceed along differentiation pathways and initiate specific genetic programs, their capacity for division diminishes (Congdon & Reya, 2008). External information combined with internal preparedness are key components determining cell fate decisions. Cell cycle machinery is intimately involved in this process because it coordinates and interprets incoming signals to decide whether to proliferate or adopt an alternative fate. This decision is called the Restriction point, and its disruption is an essential event in cellular transformation (Blagosklonny & Pardee, 2002).

A major challenge for the cell is properly interpreting external signals, establishing and maintaining connections between signal and cell cycle systems, and maintaining control of proliferative potential. For example, withdrawal of growth factors or anti-mitogenic signals will cause a tissue culture cell to exit the proliferative cycle and enter a quiescent state (Zetterberg, *et al.*, 1995). Such cells can be induced to re-enter the cell cycle by growth factor addition, which rapidly activates signal transduction cascades that communicate this information to the nucleus (Pomeroy, 2009). A key pathway in this regard is Ras/Map kinase, which transmits a proliferative signal to the nucleus that jump-starts the cell cycle machinery and initiates the gene expression program required for cell duplication (Chang, *et al.*, 2003, Coleman, *et al.*, 2004, Takuwa & Takuwa, 2001). In addition, it is imperative that the nucleus alert signaling systems that their information has been received and properly acted upon. An example of such feedback will be discussed in more detail below. The Ras/Map kinase pathway and its regulation of cell cycle machinery play a key role in initiation and development of pancreatic cancer (Caldas & Kern, 1995, Moskaluk, *et al.*, 1997). Understanding normal functioning of the major cell cycle components and their connection to signal transduction pathways is therefore essential to elucidating how and why they are disrupted in the disease state.

4.2 Cell cycle machinery

Regulation of cell cycle progression is designed to ensure DNA replication and chromosome segregation occur in response to the proper signals, proceed in the required temporal order, and are carried out accurately (Suryadinata, *et al.*, 2010). The cell cycle machinery that control events can be promoters, inhibitors, or evaluators of cell cycle progression but in all cases are responsive to internal and extracellular signaling pathways (Novák, *et al.*, 2010). The six major types of activity regulated by this machinery include: 1) Establishment of ordered biochemical pathways responsible for sequential progression through the cycle; 2) Assembly/disassembly of required structures (e.g. formation of DNA origins of replication, transcription start sites, chromosome segregation sites, telomeres, etc); 3) Regulation of nanomachines controlling production (DNA/RNA polymerases, ribosomes, lipid production etc); 4) Communication of outcomes (e.g. informing signaling components that transmitted information has been received); 5) Monitoring fidelity of ongoing or completed tasks (e.g. mechanisms ensuring cell cycle events are carried out in an accurate and timely fashion); 6) Self-regulation of activities (e.g. cell cycle components often regulate themselves or each other to drive cell cycle transitions and maintain ordered progression).

Cyclin-Dependent Kinases (CDKs) phosphorylate specific protein substrates at serine/threonine residues to initiate specific events (e.g. DNA replication) and drive cell cycle transitions (Malumbres & Barbacid, 2005). Regulating CDK activity is therefore crucial, as indicated by the multiple distinct and redundant pathways controlling its function. The CDK subunit alone lacks kinase activity, so it must bind a cell cycle-specific cyclin subunit and undergo both phosphorylation and dephosphorylation at unique sites to be activated (Harper & Adams, 2001). Functional cyclin-CDK complexes can be inhibited by phosphorylation/dephosphorylation, cyclin degradation, and by tight binding of small inhibitory proteins such as members of the CIP/KIP family (p21^{CIP1}, p27^{KIP1} and p57^{KIP2}) and the INK family (p15^{INK4b}; p16^{INK4a}; p18^{INK4c} and p19^{INK4d}) (Ekholm & Reed, 2000, Morgan, 1997, Pavletich, 1999, Sheaff, 1997, Wang, Q., *et al.*, 2011a). The CDK inhibitors are typically

thought to function as tumor suppressor proteins (Sherr, C. J. & Roberts, 1995). Genetic analysis of pancreatic cancer reveals both cyclin and CDK inhibitors are commonly disrupted in the disease, either directly or by alteration of upstream signaling pathways (Chen, Jinyun, *et al.*, 2009b, Gansauge, *et al.*, 1997, Kornmann, Marko, *et al.*, 1998a, Lee, *et al.*, 2009, Schutte, *et al.*, 1997).

4.3 The restriction point

The Restriction point represents an operationally defined transition in G1 when the cell decides whether to proceed with the proliferative cycle or withdraw and adopt an alternative fate (Blagosklonny & Pardee, 2002). The cell cycle machinery makes the decision based on evaluation of external signals and internal preparedness. Before the Restriction point, cell cycle progression is dependent on mitogen stimulation and thus represents a period in which the cell is still receiving information and evaluating its ability to successfully divide (Blagosklonny & Pardee, 2002, Sheaff & Roberts, 1998). After the Restriction point, cell cycle progression no longer requires growth factor stimulation and the cell is committed to completing the proliferative cycle (Blagosklonny & Pardee, 2002, Sheaff & Roberts, 1998). Mitogen signaling performs three main functions: 1) It establishes and maintains extracellular contact with the cell, transmitting the need for duplication; 2) It activates and communicates with cell cycle machinery to drive progression through the cycle; and 3) It initiates the gene expression programs required for cell duplication.

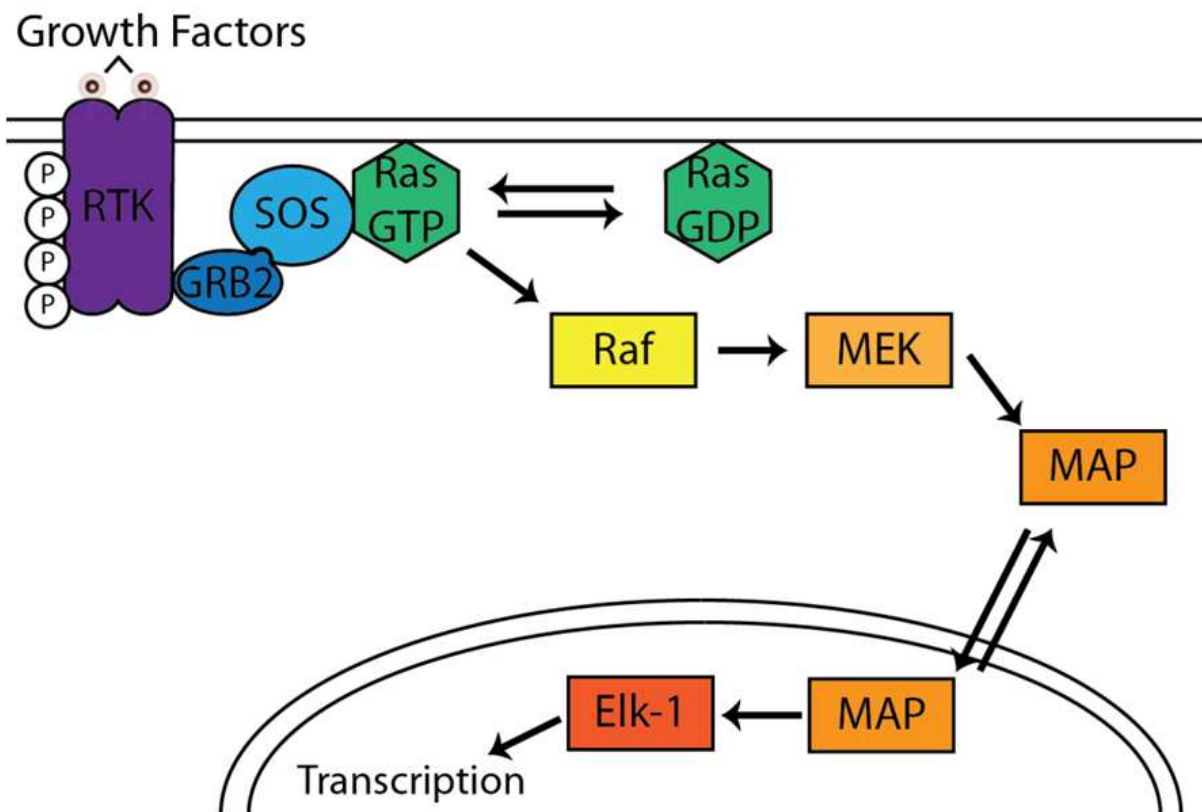


Fig. 4. **The Ras/Map Pathway.** External growth factors bind to the receptor tyrosine kinase (RTK) and initiate the transduction of the signal down the protein chain, the end result of which is the transcription of factors required for initiation of the cell cycle and cellular division.

To elucidate the molecular events describing the Restriction point, recall how tissue culture cells exit the cell cycle in response to mitogen withdrawal. Re-stimulating these quiescent cells with growth factors causes a rapid activation of various signal transduction cascades, including the Ras/Map kinase pathway (see Figure 4). In normal cells, this process typically involves transient accumulation of active Ras-GTP, which presumably reflects successful communication with the nucleus. While growth factor binding to receptor tyrosine kinases is responsible for the rapid accumulation of Ras-GTP, it does not explain the transient nature of Ras activation. Work from our lab suggests activation of the Map kinase cascade initiates a negative feedback loop that indicates successful transmission and so prevents further Ras activation (Moeller, *et al.*, 2003).

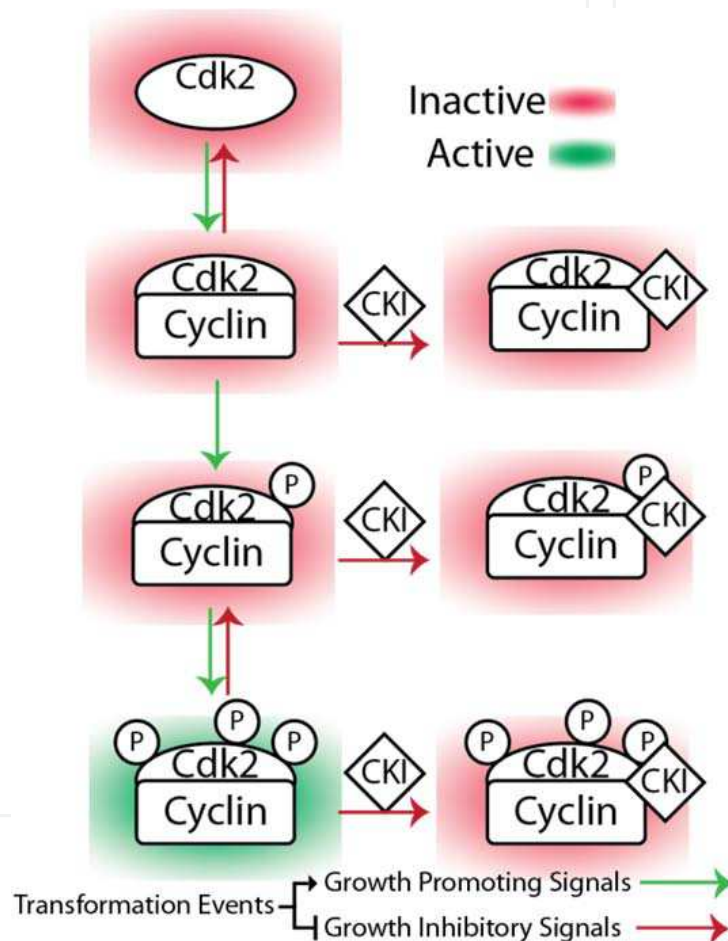


Fig. 5. **CDK2 Regulation.** Mitogen stimulation eventually leads to the activation of Cdk2, an important late G1 and S phase regulator through the production and subsequent binding of cyclins followed by phosphorylation of the complex. Inhibitory signals can lead to dephosphorylation of the complex, the degradation of the complex, or it can be bound by cyclin kinase inhibitors (CKI).

Map kinase is translocated into the nucleus, where it initiates transcriptional programs required for cell cycling and duplication. It also phosphorylates the CDK2 inhibitor p27kip1, resulting in its export from the nucleus. Cytoplasmic p27 targets GRB2 and prevents its interaction with the guanine nucleotide exchange factor SOS, thereby preventing formation of the GRB2-SOS complex that recruits Ras to the membrane. These observations led us to

hypothesize that p27 plays an important role in a negative feedback loop ensuring that: 1) The successful transmission of external information to the nucleus is communicated to the signaling system; 2) The magnitude and duration of the signal is properly maintained and regulated to prevent activation of fail-safe checkpoints and/or hyperproliferation. As will be discussed below, our more recent evidence suggests disruption of this pathway could be relevant to the initiation and progression of pancreatic tumorigenesis.

In addition to opening lines of communication, an early response to growth factor stimulation is synthesis of cyclin D and activation of CDK4/CDK6 (Ladha, *et al.*, 1998, Ohtsubo & Chibazakura, 1996). Cyclin D-CDK4/6 phosphorylates the retinoblastoma protein (pRb), a well known tumor suppressor that binds and inhibits the transcription factor E2F (Knudsen & Knudsen, 2008). As a result, E2F is released and mediates transcription of genes whose products are required for cell cycle progression, mass increase, and DNA replication (Chen, Hui-Zi, *et al.*, 2009a, Nevins, 2001). Activity of cyclin D-CDK4/6 complexes is negatively regulated by the tight binding inhibitor p16 (Sherr, C. J. & Roberts, 1999, Wang, Q., *et al.*, 2011a). In pancreatic cancer, cyclin D1 overexpression and p16 inactivation are very common events, emphasizing the importance of disrupting G1 progression to disease development (Chen, Jinyun, *et al.*, 2009b, Fry, *et al.*, 2008, Gansauge, *et al.*, 1997, Kornmann, Marko, *et al.*, 1998a, Schutte, *et al.*, 1997). A major goal of E2F liberated by cyclin D-CDK4/6 is synthesizing cyclin E, which binds and activates CDK2 to continue progression through G1 and prepare for the S phase transition (Roberts & Sherr, 2003, Sheaff & Roberts, 1998). Cyclin E-CDK2 further phosphorylates pRb and releases E2F to make more cyclin E, thus establishing a positive feedback loop. The result is a burst of cyclin E-CDK2 activity that is thought to drive the transition from mitogen-dependent to mitogen-independent cell cycle progression (Sheaff & Roberts, 1998).

It will be argued below that compromised Restriction point control is the major focus of genetic alterations in pancreatic cancer development.

Another positive feedback loop is established as cells approach the G1/S phase transition. Cyclin E-CDK2 phosphorylates and inactivates its own inhibitor, p27kip1, resulting in a burst of cyclin E-CDK2 activity (Sheaff, *et al.*, 1997). As a consequence cyclin A-CDK2 is generated to propel cells into S phase and monitor its progression (Woo & Poon, 2003). After accomplishing its goals, cyclin E-CDK2 initiates its own destruction by phosphorylating cyclin E and targeting it for proteasomal degradation (Clurman, *et al.*, 1996). Once DNA is replicated, the cell transitions into G2 where the accuracy of DNA synthesis is evaluated and the cell prepares for chromosome segregation (Clarke & Gimenez-Abian, 2000). Cyclin B-CDK1 is in control during these processes (Kishimoto & Okumura, 1997). After division the system resets and cells must again evaluate internal and external signals to decide whether to continue the proliferative cycle or withdraw and adopt an alternative fate (Sheaff & Roberts, 1998). In the case of transformed cells, the default decision is generally proliferation.

5. Cell cycle machinery in development and progression of pancreatic cancer

The previously discussed hallmarks of cancer—self-sufficiency in growth signals, insensitivity to growth-inhibitory factors, unlimited replicative potential, evasion of apoptosis, angiogenesis, and metastasis—identify the biological processes which must be genetically altered to generate a transformed pancreatic cell (Hanahan & Weinberg, 2000).

As the discussion of cancer cell origins hopefully made clear, however, differentiated quiescent cells can sometimes acquire some of these characteristics during normal organism functioning. If such a facultative progenitor cell initiates pancreatic cancer, then genetic alterations likely affect the remaining uncompromised biological systems. This section will argue that a major consequence of genetic mutations in pancreatic cancer is accelerating G1 progression and disrupting Restriction point control via deregulation of the cell cycle machinery. Proliferative control mechanisms can be disrupted in one of two ways: 1) Directly, by mutation of the machinery itself; 2) Indirectly, by disruption of upstream signaling pathways. As is always the case with cancer, enhanced genetic instability underlies accumulation of transforming mutations (Negrini, *et al.*, 2010). One such genetic alteration occurring very early in pancreatic cancer is telomere abnormalities (Gisselsson, *et al.*, 2001, Hong, *et al.*, 2011, Kobitsu, *et al.*, 1997, van Heek, *et al.*, 2002).

5.1 Telomere abnormalities

Most cells have limited replicative potential determined by the rate of telomere loss (Hornsby, 2005). Chromosome ends present a special challenge to DNA replication, since the directionality of DNA polymerase and the subsequent removal of RNA primers means that genetic material is lost each round unless special precautions are taken (Gilson & Géli, 2007). The resulting ends become “sticky” and potentially interact, resulting in aberrant recombination events and chromosome breakage during anaphase. This repeated cycle of chromosome fusion and breakage has been implicated in genetic abnormalities such as amplifications and deletions that contribute to transformation (Murnane, 2010). Chromosome ends are therefore protected by TTAGGG repeats which are enzymatically added to the ends by an enzyme called telomerase (Osterhage & Friedman, 2009).

In many types of cancer telomerase is inappropriately activated in order to maintain the ends and prevent cell cycle exit (Artandi & DePinho, 2010). In the case of pancreatic cancer, however, telomere shortening appears to be a very early event in the formation of PanIN precursor lesions (Bogenrieder & Herlyn, 2003, Hong, *et al.*, 2011). It results in greatly enhanced genomic instability that causes global genome rearrangements and facilitates accumulation of subsequent point mutations. Telomerase appears to be re-activated if these lesions progress and become ductal adenocarcinomas, perhaps to reduce genome rearrangements that threaten cancer cell viability (Hong, *et al.*, 2011). The early onset of telomere shortening in pancreatic cancer suggests that the affected cell may not be subject to limited replicative potential, further evidence supporting a stem cell origin for the disease.

5.2 Mutational activation of ras signaling

K-ras appears to be the major proto-oncogene mutated in pancreatic cancer (~90% of cases), acting as an initiating event occurring very early in pre-cancerous lesions (Caldas & Kern, 1995, Moskaluk, *et al.*, 1997). The high likelihood of K-ras disruption strongly suggests that it is also disrupted in the remaining 10% of cases, albeit by different mechanisms. As will be discussed below, work from our laboratory on the CDK inhibitor p27kip1 has identified a novel pathway by which this could occur. Ras is a member of the GTPase family that plays a key role in receiving and transmitting extracellular signals to the nucleus, where they modulate gene expression and make cell fate decisions (Takuwa & Takuwa, 2001). It is

recruited to the membrane upon activation of receptor tyrosine kinases by association with GRB2-SOS (Chang, *et al.*, 2003). This complex activates Ras by converting it to the GTP-bound form, which initiates signal transmission via activation of the Map kinase cascade (see Figure 4) (Coleman, *et al.*, 2004). The ability to turn off signaling is crucial, so Ras is a GTPase that can hydrolyze GTP to GDP (Bernards, 2003). Ras regulation therefore centers in large part on controlling GTP hydrolysis and GDP dissociation to achieve the proper degree and duration of downstream signaling.

Sequencing of primary pancreatic cancer samples revealed that K-ras mutations tend to target codon 12 and inactivate GTPase activity (Caldas & Kern, 1995, Moskaluk, *et al.*, 1997). The resulting K-ras-GTP continues downstream signaling in the absence of upstream effectors and in spite of inhibitory signals. Although mutated K-ras is in its active GTP bound form, it may still need to be localized to the membrane in order to initiate downstream signaling (Weise, *et al.*, 2011). Thus, other regulatory events help determine the level and duration of downstream signaling from mutated K-ras. For this reason, additional mutations affecting Ras regulation are also observed. These results illustrate how disrupting key signaling pathways requires the synergistic effects of multiple genetic disruptions due to fail-safe mechanisms and checkpoints that have evolved to prevent cell transformation (Hanahan & Weinberg, 2000).

Signaling pathways affected to be activated by mutant K-ras are the Raf-Map kinase cascade, PI3K-AKT, and RalGDS, with each making a distinct contribution to development of the transformed cell (Calvo, *et al.*, 2010). As discussed above, Ras/Map kinase plays a key role in promoting cell cycle re-entry and progression. PI3K-AKT is involved in cell survival, while RalGDS is one of several Ras-regulated guanine-nucleotide exchange factors that activates Ral A and B GTPases (Carnero, *et al.*, 2008, Ferro & Trabalzini, 2010). Ral proteins regulate key cellular processes such as endocytosis, exocytosis, and actin organization, as well as contributing to regulation of gene expression (Carnero, *et al.*, 2008, Ferro & Trabalzini, 2010). A number of additional genetic disruptions appear to be required for mutant K-ras induced transformation. Of particular interest is excess cyclin D1, itself a downstream target of Ras that is commonly mutated in the disease state (Fry, *et al.*, 2008, Gansauge, *et al.*, 1997, Kornmann, Marko, *et al.*, 1998a). Given the pleiotropic effects of Ras signaling, further work is required to completely describe its contribution to transformation.

5.3 Mutational activation of other proto-oncogenic signaling pathways

The Ras/Map kinase pathway is only one of twelve core signaling pathway disrupted in the disease (Jones, *et al.*, 2008). It is beyond the scope of this chapter to discuss each pathway in detail, other than to note that the consequences of their disruption likely mimic, enhance, or synergize with K-ras mutations to drive transformation and cancer progression. Involvement of Notch and hedgehog signaling deserves special mention, as these critical pathways are best known for maintaining cells in an undifferentiated state during development (Kelleher, 2011, Ristorcelli & Lombardo, 2010). In the adult organism, these pathways are involved in tissue homeostasis via maintenance of tissue stem cell populations. During injury, there is a transient induction and expansion of Hedgehog or Notch dependent stem cell populations to replace damaged or lost cells (Siveke, *et al.*, 2008). In the mature pancreas, Notch signaling is restricted to centroacinar cells, suggesting they

might be the originating cancer cell (Miyamoto, *et al.*, 2003). Support for this idea comes from mice with conditional knockout of the PTEN tumor suppressor in the pancreas, which develop ductal metaplasias resulting from expansion of CACs (Hill, *et al.*, 2010). Similarly, expression of Notch components is elevated in PanIN lesions and invasive cancer (Wang, Zhiwei, *et al.*, 2011b). Mutational activation of these pathways cooperates with K-ras mutations throughout development of pancreatic cancer, from generating precancerous PanIN lesions to involvement in tumor maintenance and metastases. Both the Hedgehog and Notch pathways appear to be disrupted by ligand overexpression rather than direct mutational targeting of pathway constituents.

5.4 Mutational inactivation of tumor suppressors

Temporal disruption of specific tumor suppressors is also observed in the PanIN progression model and again seem to converge on enhancing genomic instability and/or disrupting Restriction point control. For most cancers, mutations in the tumor suppressor p53 or one of its regulatory components (e.g. ARF) are present in the majority of cases (Sherr, C. J., 1998). p53 is the central transcriptional regulator responding to all types of cell stress. It induces expression of proteins to stop the cell cycle and determine if damage can be repaired; if not, it helps activate the apoptotic pathway (Muller, *et al.*, 2011). Inactivation of this pathway is so common because it contributes to cell immortalization and allows accumulation of further genetic mutations (Hanahan & Weinberg, 2000). It is somewhat curious that p53 mutations are observed in only 50% of pancreatic cancers, and they tend to occur later in the progression model (Morton, *et al.*, 2011). This observation (as was the case with telomere shortening), suggests the initiating cell might already be immortal or at least more resistant to apoptosis. Mutation of p53 could therefore contribute something else to development of pancreatic cancer. A central target of p53 is p21cip1, a CDK2 inhibitor that blocks cell cycle progression in G1 phase (Doucas, *et al.*, 2006, el-Deiry, 1998). Thus, its mutation could enhance the rate of G1 progression and passage through the Restriction point.

The p16INK4A CDK inhibitor specifically targets cyclin D and is inactivated in >80% of pancreatic cancers (Gansauge, *et al.*, 1997, Kornmann, Marko, *et al.*, 1998a). This suggests enhanced activity of cyclin D-CDK4/6 complexes makes an important contribution to development of pancreatic cancer, consistent with the common overexpression of cyclin D1 (Fry, *et al.*, 2008). The mechanism of p16 loss is diverse, involving homozygous deletion (40%), intragenic mutation followed by inactivation of the second allele (40%), and methylation inactivation of the promoter (10-15%) (Gansauge, *et al.*, 1997, Kornmann, Marko, *et al.*, 1998a). The locus encoding for this gene is unique in that it also encodes for p14ARF, an alternative reading frame gene product that plays a key role in activating p53 by preventing its degradation (Sherr, C. J., 2001). Specific inactivation of p16 plays a greater role in pancreatic cancer than loss of ARF function, as mutations have been characterized that compromise p16 while leaving ARF intact (Jeong, *et al.*, 2005, Maitra, A & Hruban, 2008). As discussed above, this observation is intriguing since loss of ARF is the major alternative pathway for inactivating p53 (Sherr, C. J., 2001). Preferential targeting of p16 further illustrates the importance of compromising Restriction point control via disruption of the cyclins and CDKs controlling G1 progression.

Other identified mutations support this idea. SMAD4 is a cytoplasmic transcription factor involved in G1 cell cycle arrest mediated by the TGF β pathway (Yang, Guan & Yang, 2010). It is mutated ~50% of pancreatic cancers (Blackford, *et al.*, 2009). Its normal function involves association with SMAD2/3 and translocation into the nucleus where it mediates transcriptional activation of growth inhibitory genes like p27kip1 (Yang, Guan & Yang, 2010). Again, these results can be interpreted as modulating the cell cycle machinery controlling G1 progression and the Restriction point.

5.5 Animal models of pancreatic cancer

Genetically engineered mice have been developed in which genes of interest can be expressed in the pancreas using organ-specific promoter constructs. Mutant K-ras expression in the mature ductal epithelium gave little phenotype and no precursor lesions, suggesting these are not the cells of origin (Brembeck, *et al.*, 2003). However, the K-ras mutation was able to induce carcinogenesis when combined with chemically induced pancreatic injury (Guerra, *et al.*, 2007). Under these conditions mature differentiated cells are directed to re-enter the cell cycle to replace lost cells. This result strongly supports the idea of a stem cell or facultative progenitor cell as the originating cancer cell.

In contrast, mice developed a spectrum of precursor lesions when oncogenic K-ras was expressed from its endogenous promoter in the developing pancreas (Guerra, *et al.*, 2007). Only 10% developed into metastatic adenocarcinoma, however, and it required a long latency period. Similar results were observed when mutant K-ras was specifically expressed during development in acinar cells under control of the elastase promoter (Hruban, *et al.*, 2006). Ras-induced senescence may be responsible for the lack of tumor formation, underscoring the importance of cooperating mutations (Dimauro & David, 2010). This hypothesis was confirmed by the much greater penetrance in mice with combinations of mutations (K-ras plus p53 or p16) (Wang, Zhiwei, *et al.*, 2011b). Under these conditions, the putative cell of origin responds to mutated K-ras by differentiating along a ductal lineage (hence the ductal adenocarcinomas). Inactivating the tumor suppressors p16 or p53 alone did not give an obvious phenotype or precursor lesions, suggesting K-ras acts as an initiator mutation (Wang, Zhiwei, *et al.*, 2011b). Similarly, conditional Smad4 or TGF β receptor deletions were not sufficient to induce mPanIN lesions or cancer, although they clearly cooperated with K-ras to induce pancreatic neoplasia (Bardeesy, *et al.*, 2006).

5.6 Centrality of disrupted restriction point control

Altered G1 progression and Restriction point control appear to be key events in development of pancreatic cancer based on mutations involved and the putative cell of origin. The central role of cell cycle disruptions can be modeled by considering mutations in the context of the progression model. Greater than 90% of low-grade PanIN lesions show shortened telomeres, providing a mechanism for rapidly generating genetic alterations required for cell transformation (Hong, *et al.*, 2011, van Heek, *et al.*, 2002). K-ras mutation is one of the earliest abnormalities and is likely an initiating event, being present in 36% of PanIN-1A, 44% of PanIN-1B, and 87% of PanIN-2/3 precursor lesions (Caldas & Kern, 1995, Moskaluk, *et al.*, 1997). That such a dramatic alteration occurs so early is interesting, since expressing mutated K-ras in animal models typically results in senescence or apoptosis as a

protective response to loss of proliferative control (Dimauro & David, 2010, Overmeyer & Maltese, 2011). Taken together, telomere shortening and K-ras activation point to the initial transformation involving a stem cell or facultative progenitor that re-enters the cell cycle in response to injury/tissue maintenance.

Such cells likely already possess characteristics of cancer cells such as immortality and replicative potential. Thus, mutations are not required to drive cells back into the proliferative cycle from a quiescent state. Their predisposition to immortality means tumor suppressors like p53 or ARF need not be immediately inactivated, while telomere shortening is tolerated and beneficial since it induces genomic instability. What then is the limiting factor in transformation and expansion of the cancer cell? It is becoming increasingly apparent that the kinetics of stem cell cycling are quite different than those of somatic cells in culture (Nacusi & Sheaff, 2007, Neganova & Lako, 2008). Although they have limitless replicative capacity, stem cells generally take much longer to duplicate (i.e. doubling time of days). If the initiating cell in pancreatic cancer replicates so slowly, a major consequence of K-ras mutation might be to speed up the duplication rate. The overall time required for cell cycling is determined in large part by the rate of G1 progression (the longest phase of the cell cycle), so mutations would be expected to speed up this process and ensure passage through the Restriction point (Salomoni & Calegari, 2010).

One way mutant K-ras might increase the proliferative rate is by increasing activity of cell cycle components involved in the rate limiting step(s) for G1 progression (see Figure 7). Experiments in tissue culture cells suggest that overexpressing G1 cyclins or otherwise increasing CDK activity (e.g. blocking its inhibition) can accelerate G1 phase (Roberts & Sherr, 2003, Sherr, C. J. & Roberts, 1999). Similarly, cyclin D1 is commonly overexpressed and its inhibitor p16 inactivated in pancreatic cancers (Chen, Jinyun, *et al.*, 2009b, Fry, *et al.*, 2008, Gansauge, *et al.*, 1997, Kornmann, Marko, *et al.*, 1998a, Lee, *et al.*, 2009, Schutte, *et al.*, 1997). Experiments using pancreatic cancer cell lines show blocking activity of excess cyclin D retarded cell growth and reduce their ability to generate tumors in nude mice (Kornmann, M., *et al.*, 1998b). Various drugs (e.g. celecoxib, green tea constituents) displaying efficacy against pancreatic cancer cell lines often target excess cyclin D (Tseng, *et al.*, 2002). Disruptions during the early stage of mitogen dependent cell cycle progression leading up to the Restriction point therefore appear critical to development of pancreatic adenocarcinoma. One obvious consequence would be to enhance cyclin D/CDK4/6 activity, suggesting Rb phosphorylation and inactivation is crucial to transformation of the initiating cell. While these early events could certainly accelerate G1 progression, the transition to mitogen independence reflected in the Restriction point is also likely to be rate limiting. Thus, cyclin E-CDK2 activity is likely to be enhanced given its role in this process (see Figure 7) (Sheaff & Roberts, 1998). Evidence in support of this hypothesis comes from analyzing the contribution of K-ras mutation on proliferation of pancreatic cancer cell lines.

Using small molecule inhibitors of MEK, Gysin *et al.* found that cells arrested in G1 due to increased expression of the tumor suppressor p27kip1 (Gysin, *et al.*, 2005). As a consequence, CDK2 was inhibited, Rb was not phosphorylated, and the E2F activity required for G1 progression was absent. These results indicate that inhibiting p27 expression is a major role of K-ras mutation, thereby enhancing CDK2 activity and accelerating G1 progression. As described below, we propose that an additional rationale for decreasing p27 is to ensure continued activation of the mutated K-ras pathway driving transformation. In

contrast, MEK inhibition had little effect on CDK4 activity, suggesting K-ras mutation is not responsible for effects on early G1 progression (Gysin, *et al.*, 2005). The obvious candidates for affecting this period are the aforementioned cyclin D1 overexpression and inactivation of p16. It should be noted that there are also mechanisms through which increasing cyclin D1 levels could contribute to enhanced CDK2 activity. Additional support for the importance of CDK2 comes from analysis of OGF signaling, which negatively regulates progression of pancreatic cancer (Fan, *et al.*, 2008). It does so by inducing expression of the CDK2 inhibitor p21cip1, further evidence that accelerating G1 progression is a key step in disease development (Fan, *et al.*, 2008).

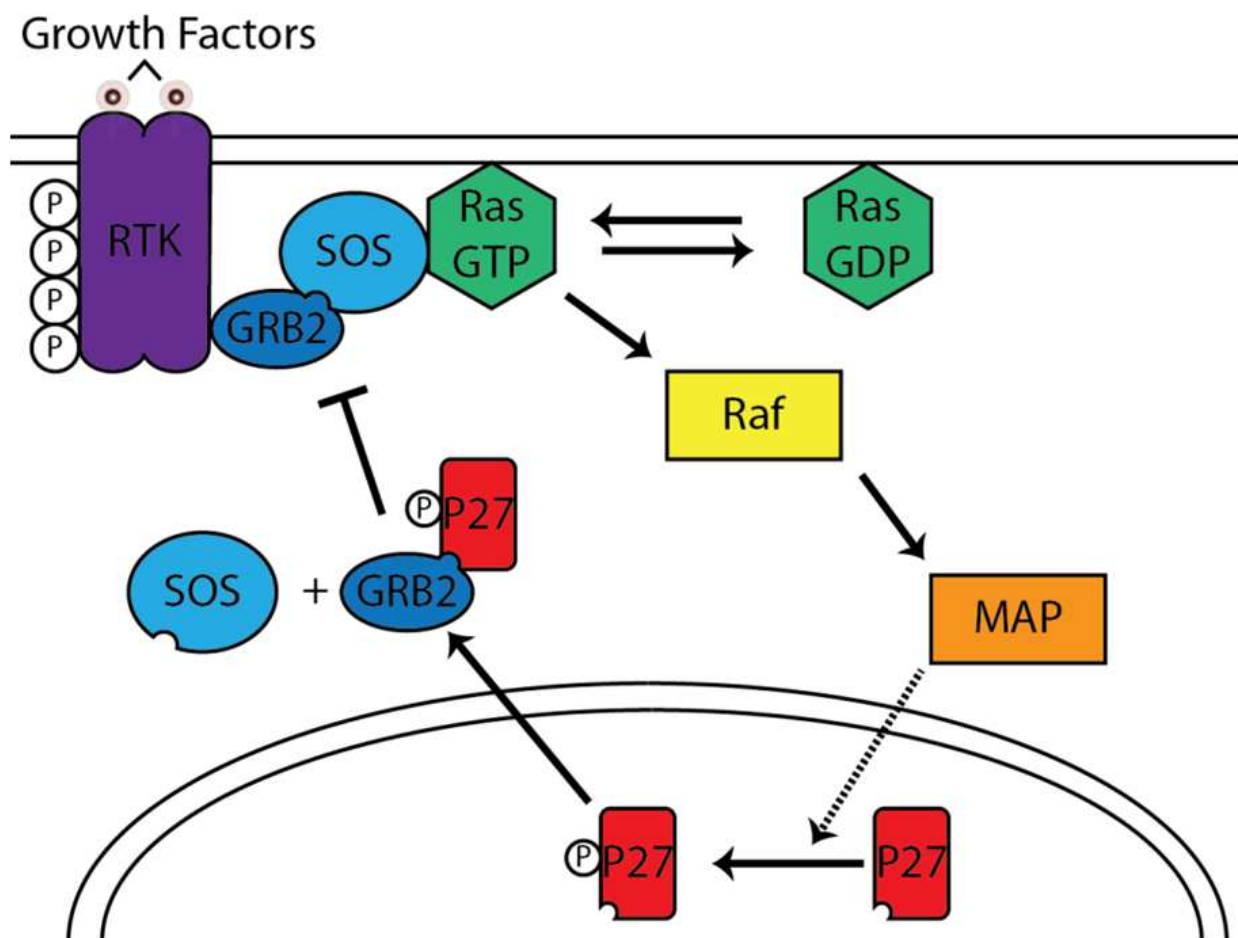


Fig. 6. **Ras/Map Regulation by p27.** Mitogen stimulation of the Ras/Map pathway leads to the phosphorylation and export of p27 from the nucleus. This exported P-p27 binds competitively for Grb2 against SOS. The dissociation of the Grb2/SOS complex leads to the down regulation of the Ras/Map pathway signal.

In addition to its effects on early G1 phase, overexpressed cyclin D1 is known to bind and sequester p27 (Sherr, C. J. & Roberts, 1999). This would indirectly enhance CDK2 activity and hence progression through the Restriction point. Work in our lab has shown that p27 also functions in a negative feedback loop regulating Ras activation by GRB2 (see Figure 6) (Moeller, *et al.*, 2003). If cyclin D1 were to sequester p27, this negative feedback would be disrupted, thereby contributing to sustained K-ras signaling and accelerated cell cycle

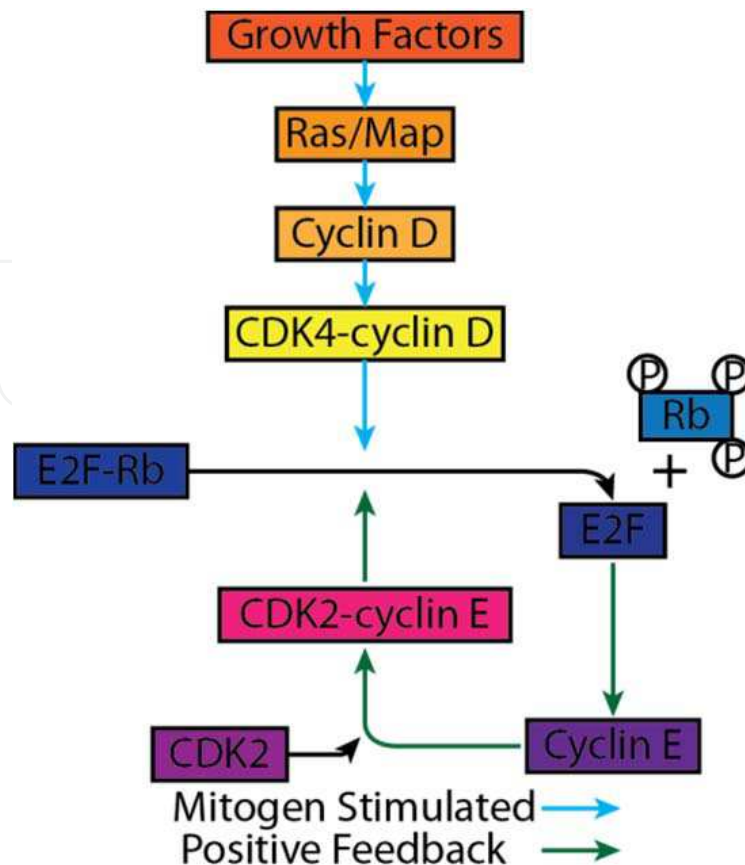


Fig. 7. **E2F Activation and Positive Feedback.** Mitogen stimulation leads to the phosphorylation and removal of the cell cycle regulating protein Rb from the E2F-Rb complex. E2F goes on to activate cyclin E, whose complex with CDK2 further activates more E2F by Rb phosphorylation. This self-sustained positive feedback marks the transition from mitogen stimulated to self-sustained cell cycle progression.

progression. We have recently investigated this possibility in breast cancer cells (which often overexpress cyclin D1) and found that it preferentially sequestered the phosphorylated p27 that is exported to inhibit GRB2 (submitted). We are now investigating whether the excess cyclin D1 in pancreatic cancer cell lines causes a similar disruption in p27 regulation of the Ras pathway. These observations could help explain why cyclin D1 overexpression is necessary for the oncogenic effects of K-ras in pancreatic cancer (Fry, *et al.*, 2008). Given that the activation of facultative progenitor cells in response to injuries is normally transient, inappropriate K-ras signaling could also play an important role in preventing their withdrawal from the cell cycle. This hypothesis is consistent with results showing that constitutive K-ras activation is required for maintenance of pancreatic cancer (Caldas & Kern, 1995).

6. Conclusions

Given the aggressiveness and poor prognosis of pancreatic cancer compared to many other forms of the disease, it is worthwhile to consider what makes it unique. This chapter provides an evaluation of mutational changes and disrupted biological function within the context of cancer hallmarks and identity of the originating cell. We argue that mutations

directly and indirectly affecting G1 progression and the Restriction point are crucial to development of the pancreatic cancer cell. This analysis will hopefully stimulate further research into methodologies for treating the disease by targeting disruptions in or activity of the relevant cell cycle machinery. This approach may offer greater specificity and fewer side effects than previously expected. Increasing evidence provides tantalizing clues that proliferative control mechanisms in normal and transformed cells might be different (Moeller & Sheaff, 2006). Mice lacking cyclins and CDKs, for example, can still develop normally (Sherr, Charles I. & Roberts, 2004). The dispensability of some cell cycle machinery in normal development and untransformed cells is in stark contrast to its apparent necessity in pancreatic cancer. Thus, the cell cycle machinery and its activities may represent viable therapeutic targets with unanticipated specificity for preferentially inhibiting proliferation of the pancreatic cancer cell.

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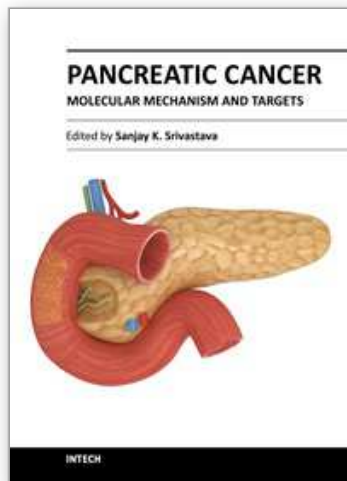
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This book provides the reader with an overall understanding of the biology of pancreatic cancer, hereditary, complex signaling pathways and alternative therapies. The book explains nutrigenomics and epigenetics mechanisms such as DNA methylation, which may explain the etiology or progression of pancreatic cancer. Book also summarizes the molecular control of oncogenic pathways such as K-Ras and KLF4. Since pancreatic cancer metastasizes to vital organs resulting in poor prognosis, special emphasis is given to the mechanism of tumor cell invasion and metastasis. Role of nitric oxide and Syk kinase in tumor metastasis is discussed in detail. Prevention strategies for pancreatic cancer are also described. The molecular mechanisms of the anti-cancer effects of curcumin, benzyl isothiocyanate and vitamin D are discussed in detail. Furthermore, this book covers the basic mechanisms of resistance of pancreatic cancer to chemotherapy drugs such as gemcitabine and 5-flourouracil.

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