Defining the Cancer Master Switch

Courtney J. Balentine · David H. Berger · Shi-He Liu · Changyi Chen · John Nemunaitis · F. Charles Brunicardi

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Abstract
Background Recent research has focused on signaling cascades and their interactions yielding considerable insight into which genetic pathways are targeted and how they tend to be altered in tumors. Therapeutic interventions now can be designed based on the knowledge of pathways vital to tumor growth and survival. These critical targets for intervention, master switches for cancer, are termed so because the tumor attempts to “flip the switch” in a way that promotes its survival, whereas molecular therapy aims to “switch off” signals important for tumor-related processes.

Methods Literature review.

Conclusions Defining useful targets for therapy depends on identifying pathways that are crucial for tumor growth, survival, and metastasis. Because not all signaling cascades are created equal, selecting master switches or targets for intervention needs to be done in a systematic fashion. This discussion proposes a set of criteria to define what it means to be a cancer master switch and provides examples to illustrate their application.

Introduction

Advances in technology for assessing genomic changes have resulted in the rapid expansion of our understanding of tumor biology [1–3]. Research interest currently is shifting away from the study of individual genes toward that of pathways and networks because of the increased ability to conduct rapid and unbiased surveys of the genome for mutations, chromosomal rearrangement, epigenetic phenomenon, and other changes affecting gene expression [4–7]. Focusing on signaling cascades and their interactions rather than individual genes has yielded considerable insight into which genetic pathways are targeted and how they tend to be altered in tumors [8–10]. Therapeutic interventions can be designed based on the knowledge of pathways vital to tumor growth and survival. These critical targets for intervention can be thought of as master switches for cancer, with the tumor attempting to “flip the switch” in a way that promotes its survival, whereas molecular therapy aims to “switch off” signals important for tumor-related processes. Defining useful targets for therapy depends on identifying pathways that are crucial for tumor growth, survival, and metastasis. Because not all signaling cascades are created equal, selecting master switches or targets for intervention needs to be performed in a systematic fashion. Toward this end, we propose a set of criteria to define what it means to be a cancer master switch and provide a few examples to illustrate their application.
Defining criteria for a master switch

Figure 1 illustrates our conceptual model for the different ways in which molecular pathways can function as a master switch for cancer. Although there are multiple ways in which a tumor can develop and spread, there are certain commonalities that persist across tumor type. A pathway initiated by the binding of ligand and receptor can promote tumor growth and invasion through activation of downstream transcription factors and regulatory proteins that modulate multiple pathways. The sum total of these interactions promotes processes important to tumor development, survival, and metastasis. The balance of stimulatory and inhibitory crosstalk between pathways is a delicate balance that the tumor upsets by carefully targeted genetic and epigenetic changes. Generally, a single change is inadequate to upset this balance so tumors tend to deregulate multiple processes involving signal activation and downstream regulation.

When determining the significance of a particular pathway, we believe it is important to demonstrate that disruption of its component signaling occurs in vivo and does not represent only an in vitro artifact. Consequently, a master switch is more than a promising mutation found in a petri dish or animal model, and must have demonstrated relevance in human disease. Tumor-induced changes can take the form of alterations in gene sequence or structure as well as epigenetic silencing through methylation or histone modification [6, 7]. A pathway constituting a master switch ought to be altered at a high frequency, because this reflects its importance in tumor evolution [5]. Moreover, these changes should have some connection to tumor genesis, survival, or metastasis. Additionally, because tumor development involves significant crosstalk between signaling cascades rather than single molecules acting in isolation, a master switch ought to communicate with other pathways that are significant for tumor development. Relationships with other important pathways can take the form of physical interaction between components of each cascade as well as transcriptional and translational regulation. Alternatively, a master switch can be involved in global regulation of mutation or transcription so that multiple pathways are simultaneously affected by changes in the master switch. To clarify the application of these criteria for their identification and targeting as cancer master switches, we offer three examples of master switch candidates.

Colorectal cancer and Wnt signaling

One of the earliest mutational events occurring in colorectal cancer is loss of adenomatosis polyposis coli (APC) expression, and this change is found in the majority of sporadic colorectal cancers [11]. APC mutation also is seen in familial adenomatous polyposis (FAP) with both point mutations and deletions playing a role [12–16]. It is not uncommon, however, to see a combination of inherited mutations in some form giving rise to the loss of most, but not all, APC expression. The concept that total loss of APC expression actually is counterproductive for tumor growth has been called the just-right signaling model that proposes that a certain level of APC expression actually is advantageous for tumor growth [17]. Moreover, there is some evidence to suggest that when mutation of the APC gene does not occur, methylation of the locus results in decreased expression [18]. APC is an important element in the Wnt-signaling pathway that plays an important role in tumor development. In canonical Wnt signaling, the transcription factor beta-catenin is bound in a cytoplasmic destruction complex involving APC, axin, GSK3β, and CK1γ [19]. In the absence of a Wnt ligand, beta-catenin is maintained in the destruction complex where it is phosphorylated by GSK3β and CK1γ [19–21]. Phosphorylated beta-catenin is targeted for proteasome degradation, and Wnt signaling remains in an inactive state. Activation of the canonical Wnt pathway involves Wnt ligand binding to Frizzled (Fz) receptors in the presence of lipoprotein receptor-related protein (LRP)5/6 and is inhibited by binding of sclerostin (SOST) or soluble Fz protein [22–27]. After the binding of Wnt to the Fz receptor complex, phosphorylation of beta-catenin via the destruction complex is inhibited. Beta-catenin is then free to translocate to the nucleus where it interacts with T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors to trigger gene expression [28–30]. In addition to the previously mentioned mutations in APC, there are several other alterations utilized by colorectal cancers to affect Wnt signaling. Mutations in beta-catenin exon 3 have been found in patients with hereditary nonpolyposis colorectal cancer, and AXIN1 mutations at GSK3β binding sites have been described as well [31–33]. When wild-type AXIN1 is present, mutations in AXIN2 have been described [34]. Additionally, epigenetic changes, including hypermethylation as well as somatic mutations of various Fz proteins, also have been found [35–37].

Elements of Wnt signaling interact with other pathways that play important roles in colorectal cancer. These pathways include transforming growth factor beta (TGF-β), mitogen-activated protein kinases (MAPK), phosphatase and tensin homolog (PTEN), and phosphatidylinositol 3-kinase (PI3 K)/Akt, which are responsible for regulating cell growth and survival [38]. These relationships tend to be exceedingly complex and are context-dependent. In some instances, Wnt signaling negatively regulates factors in the TGF or bone morphogenetic protein (BMP) pathway, but they also can cooperate to increase transcription of
common targets [39–42]. A similarly complicated interaction is observed with other pathways modulated by Wnt signaling, which makes it difficult to generalize results from one model system to another [43, 44]. Moreover, although mutations in the Wnt pathway have long been recognized as an important early event in the genesis of colorectal cancer, recent work has linked downstream products of Wnt signaling with metastasis, resistance to apoptosis, and decreased survival [45–47]. Recent evaluation of the Wnt pathway using ribonucleic acid interference (RNAi) also has shown promise in identifying potential for intervening in various cancer processes [48–55]. In short, Wnt signaling plays an integral role in the development and progression of colorectal cancer through direct activation of downstream factors and via interaction with other pathways that lead to deregulation of cell growth and survival. Therefore, the Wnt pathway represents an ideal candidate to be targeted for therapeutic and diagnostic measures.

**Lung cancer and EGFR**

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase with three other family members: ErbB-2/HER2/neu, ErbB-3/HER3, and ErbB-4/HER4 [56]. Like most tyrosine kinase receptors, ligand binding to EGFR induces hetero- or homodimerization and subsequent autophosphorylation, which leads to recruitment of downstream proteins for signaling [56–58]. Interestingly, EGFR heterodimerization with HER2 leads to stronger activation than homodimerization and this has
important therapeutic implications. One of the important pathways activated by EGFR is the Ras/Erk signaling cascade, which is involved in cell proliferation and survival [59]. Once activated, Ras leads to Raf phosphorylation and subsequent stimulation of the MAPK pathway, which also plays a critical role in cell proliferation and survival [60, 61]. The ability of human Raf to activate the MAPK pathway differs depending on each isoform [61]. Additionally, EGF signaling can activate the MAPK pathway via PKC and also can stimulate the PI3 K/Akt pathway, which is frequently altered in human tumors [62, 63]. Finally, EGF signaling promotes expression of vascular endothelial growth factor (VEGF), which is involved in angiogenesis. Overall, abnormalities in EGF signaling have been implicated in tumor proliferation, invasion, angiogenesis, and metastasis [58, 64, 65]. As one might expect from a signaling cascade that intersects with so many other pathways, overexpression of EGFR is quite common in human tumors, including lung cancer and glioblastoma [66, 67]. EGFR mutations also are common in the tyrosine kinase domain, and EGFR expression has been correlated with both disease-free and overall survival rates in lung cancer [68–70].

EGF signaling in lung cancer has become an important therapeutic target. Tyrosine kinase inhibitors, such as gefitinib, have demonstrated efficacy in clinical trials when used in combination with standard chemotherapy [71]. These agents have proven particularly effective in the presence of kinase domain mutations and in lung cancers overexpressing both EGFR and human epidermal growth factor receptor-2 (HER2) [72, 73]. Another approach targeting EGFR involves the use of monoclonal antibodies, such as Erbitux/ cetuximab, that lead to receptor internalization and degradation [56]. These agents also have shown the ability to improve survival in selected patients with tumors expressing EGFR [74]. Because the EGF pathway influences a multitude of processes in tumor development and is a promising target for intervention, we believe that it should be included in any discussion of tumor master switches.

**PDX-1, embryologic transcription factors, and pancreatic cancer**

Pancreatic and duodenal homeobox 1 (PDX-1), a member of the homeodomain-containing transcription factor family, plays a crucial role in the development of the pancreas as well as maintaining normal endocrine function of the adult pancreas [75–79]. The absence of PDX-1 results in pancreatic agenesis in mice and a compound heterozygous mutation in the PDX-1 homeodomain causes human pancreatic agenesis [80, 81]. In the adult pancreas, PDX-1 regulates multiple genes’ expression (e.g., insulin, islet amyloid polypeptide, glucokinase, and glucose transporter) [82–85]. PDX-1 also is a critical factor in mediating cell dedifferentiation [86, 87], transdifferentiation [88–91], and cell metaplasia [92, 93]. Most importantly, re-expression of PDX-1 has been found in a variety of human tumors, including those of pancreatic cancer, breast cancer, gastric cancer, and prostate cancer as well as a genetically engineered murine tumor model [93–104]. PDX-1 is capable of inducing cell proliferation, invasion, and transformation in vitro and promoting tumor development and growth in vivo, demonstrating its oncogenic properties [105]. These characteristics meet the criteria for classification as a cancer master switch [106].

Elucidation of the regulatory network of PDX-1 provides a better understanding of PDX-1 as a master switch. PDX-1 is reported to directly regulate insulin gene expression as well as the expression of the genes encoding glucose transporter 2 (Slc2a2), islet amyloid polypeptide precursor, Pax4, synaptotagmin 1, and Pdx-1 itself [82–85]. PDX-1 also negatively regulates expression of glucagon [107], which is normally expressed in pancreatic alpha cells and Keratin 19, a member of the cytokeratin family which is critical for maintenance of cellular architecture and organization [108]. In beta cells, PDX-1 binds several groups of newly identified targets to regulate gene expression, therefore, regulating cell metabolism, proliferation, and apoptosis [109]. One target is the transcription factor p53, a master regulator of cell growth arrest and cell death. In addition, micro-RNAs are targets for PDX-1. In pancreatic cancer cells, knockdown of PDX-1 expression stimulates apoptosis through a blockade of NFkB signaling, as well as arrest of the cell cycle by downregulation of cyclins. Decreased gene expression related to angiogenesis (vascular endothelial growth factor) and invasion were also demonstrated [105]. Indirect evidence also supports the suggestion that PDX-1 may be involved in many signaling pathways, such as those involving Stat3, MAPK, and PI3 K/Akt/mTOR [92]. Our group has demonstrated that overexpression of PDX-1 in pancreatic cancer cell lines resulted in disruption of the cell cycle, which caused increased cell proliferation and invasion emphasizing a critical feature of PDX-1 as the master switch in pancreatic carcinogenesis [97, 105]. Similar to PDX-1 are the sonic hedgehog homolog (SHH) [110, 111] and Notch [112] signaling pathways that belong to a set of genes required for embryogenesis. Notch and SHH have been demonstrated to be oncogenes because of their involvement in tumorigenesis and progression. It has been suggested that both Notch and SHH are cancer master switches.

In addition to its relationship with the pathways listed above, PDX-1 also interacts with the somatostatin pathway which also plays a key role in pancreatic cancer. The somatostatin signaling pathway has been shown to down-regulate the growth of pancreatic cancer [113–116].
Somatostatin receptors are G-protein-coupled receptors that control pathways responsible for cell growth and proliferation, with both SSTR1 and SSTR2 transfection into pancreatic cancer cells leading to decreased cell growth [117–119]. Somatostatin receptors also may play an important role in regulating apoptosis in combination with tumor necrosis factors (TNFs) [120, 121]. Activation of the somatostatin pathway has shown potential for use as adjuvant therapy in pancreatic cancer and has inhibited tumor growth in animal models [114, 122]. Moreover, transfection of pancreatic cancer cells with somatostatin receptor (SSTR)-1 or SSTR-2 inhibits proliferation and increases susceptibility to treatment with somatostatin analogues [117, 118]. SSTR-2 overexpression also may block pancreatic cancer metastasis [123]. PDX-1 is a downstream transcription factor involved in somatostatin expression in adult islet cells as well as pancreatic development and insulin regulation [79, 124]. A molecular network involving SST/SSTRs and endocrine regulation, as well as SST/SSTRs, PDX-1, cell proliferation and cycle regulation strongly suggest a central role of SSTRs in the regulatory network, further implying a master switch.

Conclusions

Defining what it means to be a cancer master switch is important to clarify the discussion regarding optimal targets for intervention in a variety of human cancers. Research efforts should focus on the pathways most likely to have an impact on patient outcomes and on targeting pathways that sit at the center of a network involved in tumor promotion, which are most likely to yield beneficial results. This new paradigm represents a fundamental shift from the previous focus on identifying individual mutations or changes in transcription. It is through the better understanding of how multiple cascades interact to promote tumor development that we will enhance the ability to target them appropriately and move toward improving patient outcomes.

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