Review

Cancer Research

Molecular or Metabolic Reprograming: What Triggers Tumor Subtypes?

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Abstract

Tumor heterogeneity is reflected and influenced by genetic, epigenetic, and metabolic differences in cancer cells and their interactions with a complex microenvironment. This heterogeneity has resulted in the stratification of tumors into subtypes, mainly based on cancer-specific genomic or transcriptomic profiles. Subtyping can lead to biomarker identification for personalized diagnosis and therapy, but stratification alone does not explain the origins of tumor heterogeneity. Heterogeneity has traditionally been thought to arise from distinct mutations/aberrations in "driver" oncogenes. However, certain subtypes appear to be the result of adaptation to the disrupted microenvironment caused by abnormal tumor vasculature triggering metabolic switches. Moreover, heterogeneity persists despite the predominance of single oncogenic driver mutations, perhaps due to second metabolic or genetic "hits." In certain cancer types, existing subtypes have metabolic and transcriptomic phenotypes that are reminiscent of normal differentiated cells, whereas others reflect the phenotypes of stem or mesenchymal cells. The cell-of-origin may, therefore, play a role in tumor heterogeneity. In this review, we focus on how cancer cell–specific heterogeneity is driven by different genetic or metabolic factors alone or in combination using specific cancers to illustrate these concepts. *Cancer Res; 76(18); 5195–200. ©2016 AACR.*

Introduction

Tumor heterogeneity refers to variations in genotype and phenotype between different tumors (intertumoral heterogeneity) or cells in a single tumor (intratumoral heterogeneity). The existence of intertumoral heterogeneity is well established and illustrated by the gene expression profiles used to stratify multiple cancer types, including, but not limited to, leukemias, glioblastoma, breast, pancreatic, and colorectal tumors into their molecular subtypes (1–6). However, the true extent of intratumoral molecular heterogeneity is only just being elucidated, in part, due to the recent exploitation of high-throughput genomic analyses of multiple biopsies from individual tumors or by the isolation and analysis of single cells (7). In general, heterogeneity in cancer cells can manifest itself in two key ways: first, by major genetic events such as somatic copy number aberrations (SCNA) and mutations; and second, phenotypic variations in transcript and protein expression levels, and, not insignificantly, major metabolic rewiring. These processes are mediated by, for instance, epigenetic programming.

Microenvironment, Genetic, and Metabolic Changes

The microenvironment and nutrients influence metabolic changes in tumors

Besides genetic differences, individual tumors also show differences in phenotypes including metabolism (5, 8). Malignant solid tumors frequently encounter mild to severe hypoxia (oxygen deficiency) due to insufficient tumor microvasculature quality and quantity, culminating in impaired oxidative phosphorylation (OXPHOS). This cellular stress induces changes in tumor transcription, respiration, and metabolism, promoting highly abnormal neovasculature formation and, ultimately, allowing increased cancer cell survival, proliferation, invasion, and metastasis (9). These cancer cells, independent of their oxygenation, increase glycolysis and produce more lactate (the Warburg effect; ref. 10). Instead, the Pasteur effect states that presence of oxygen would inhibit glycolysis (11), suggesting that under normoxic conditions cancer cells may prefer OXPHOS. Irrespective of these different effects, highly metastatic prostate cancer cell lines under normoxic conditions were shown to undergo glycolysis, while less metastatic lines were OXPHOS dependent (12). Similar effects have been seen in glioma cell lines (13). Although the apparent differences in energy metabolism in different tumors are attributable to their intrinsic genetic, epigenetic, and microenvironmental characteristics, they may also represent distinct subtypes (9).

An example of hypoxia directly influencing metabolic programming via gene expression is the activity of pyruvate kinase isoforms M1 and M2 (PKM1/2), which are essential energy metabolism regulators critically involved in the final stages of glycolysis. Under hypoxic conditions, cancer cells expressing only PKM2 proliferate faster than those expressing only PKM1 (14). PKM2 exists as a less active dimer and a more active tetramer, the former being highly expressed in proliferating cancer cells and allowing upstream metabolites to accumulate to meet increased nucleotide, amino acid, and serine biosynthesis needs (refer to review; ref. 15). This differential expression of PKM2 isoforms may represent distinct tumor subtypes.

Mutated metabolic genes as cancer drivers

A more self-evident entwining of metabolism and genetics is when mutations in genes encoding metabolic enzymes are a first

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cancer "hit". Isocitrate dehydrogenase 1 (IDH1) is an enzyme that converts α-ketoglutarate (α-KG) to citrate in the tricarboxylic acid (TCA) cycle (16). However, mutated IDH1 can convert α-KG to 2-hydroxyglutarate (2HG), inhibiting enzymes controlling epigenetic methylation and consequently altering global gene expression (16). *IDH1* mutations are implicated in glioma (17) and acute myeloid leukaemia (AML) pathogenesis (18) and, interestingly, mutated *IDH1* enrichment partially defines the proneural glioblastoma subtype (6). In addition, succinate dehydrogenase (SDH) and fumarate hydratase, enzyme complexes involved in TCA cycle, also have links with cancer (original articles referred to in ref. 19). Germline mutations resulting in the loss of one subunit of the SDH complex, SDHB, have been confirmed in a rare subset of renal cell carcinoma (20), and have shown association with therapeutic response to temozolomide in metastatic pheochromocytoma or paraganglioma (21).

Nonmetabolic driver mutations affect metabolism

Clearly, cancer is not only caused by mutations in genes directly involved in metabolism, and first-hit mutations in nonmetabolic genes also indirectly remodel tumor metabolism. Specific gene mutations often drive tumorigenesis in a large proportion of cases: *TP53* is the most frequently mutated gene in many cancers (22), and *KRAS* is a proto-oncogene mutated in over 90% of pancreatic ductal adenocarcinomas (PDA; ref. 23). In this latter case, advanced PDAs in a *Kras*-driven genetically engineered mouse (GEM) model are dependent on continued mutant *Kras* signaling, which stimulates glucose uptake and reprograms downstream anabolic metabolism (24). In addition, tumors with mutant *KRAS* are addicted to a noncanonical glutamine-supported metabolic pathway that drives their growth and upregulates aerobic glycolysis (25).

However, the requirement of mutant *KRAS* for tumor maintenance is heterogeneous, with well-differentiated epithelial cell lines, specifically the classical PDA subtype (see below), being more reliant on KRAS signaling (3, 26). If mutations in particular genes like *KRAS* are dominant cancer drivers in most cases of certain cancer types, what causes heterogeneity and different phenotypes in these tumors as the disease progresses?

Possible origins of tumor heterogeneity based on driver aberrations

Cancer subtypes are mainly associated with distinct first-hit driver aberrations in normal cells (refer to ref. 27); however, they can be genetic or metabolic aberrations (Fig. 1A, left and right). Nevertheless, second-hit metabolic changes could lead to phenotypic heterogeneity in several ways in less heterogeneous tumors. For example, in cases in which a single mutated gene (e.g., *KRAS* in PDA) usually initiates tumorigenesis as a first hit, we hypothesize that tumor heterogeneity is instigated by a second hit to metabolic reprogramming induced by the microenvironment (Fig. 1A, left). Inconsistent oxygenation and nutrient provision by imperfect microvasculature could lead to considerable microenvironment-based variability in different tumor regions (and between different tumors), prompting metabolic adaptation to local conditions that threaten cellular survival. For instance, metabolic reprogramming arising from obesity-related insulin resistance or excess reactive oxygen species (ROS) from mitochondrial metabolism could influence cancer cell proliferation and render them vulnerable to heterogeneity-causing mutations (refer to ref. 9). Similarly, second-hit molecular or genetic changes could lead to distinct tumor subtypes in less heterogeneous tumors. Because of the wide-ranging effects of metabolic and molecular reprogramming on phenotype, these adaptations could be the origin of different genetic and epigenetic subtypes.

Epigenetics facilitates metabolism–transcription feedback

While both molecular and metabolic heterogeneity undoubtedly exist, their origin is debatable. Although tumor diversity is well represented by molecular/genetic profiles, this does not imply that the heterogeneity is completely molecular/genetic in origin. Gene expression is dependent on many factors including epigenetic modifications that regulate chromatin structure and DNA accessibility to transcriptional machinery. Epigenetic enzymes may be modulated not only by their own expression and that of their regulators, but also by the availability of metabolites they require as substrates or cofactors (28). For example, tet methylcytosine dioxygenase 2 (TET2) and lysine demethylase 3A (KDM3A) are two epigenetic enzymes that employ the metabolite α-KG as a cofactor. In the presence of mutant IDH1, which can convert α-KG to 2HG (ref. 16; see above), 2HG competitively inhibits α-KG's binding to TET2 and KDM3A, influencing epigenetic marks (refer to ref. 29). In this way, genetics, epigenetics, and metabolism interact in a system to form a complex feedback mechanism.

Evidently, interactions between the tumor microenvironment, metabolism, and genetics are diverse and complex. The microenvironment regulates metabolic pathways via the epigenome and also influences them directly (refer to ref. 28). Genomic aberrations (e.g. mutations) can affect metabolic genes and nonmetabolic genes with indirect actions on metabolism (17). The different outcomes of these interactions are a potential source of the heterogeneity that can lead to distinct cancer subtypes.

Context-Specific Molecular and Metabolic Heterogeneity

Transcriptomic PanNET subtypes and their associated metabolic profiles

Given the potential for metabolic heterogeneity to influence cancer cell phenotypes and, by extension, tumor subtypes, it is sensible to analyze transcriptomic and metabolic profiles together when attempting to stratify patients. By jointly analyzing mRNA and miRNA transcriptomes, we recently stratified human PanNETs into three molecular subtypes with distinct metabolic profiles (5). One subtype, the "insulinoma-like tumors" (IT; with increased insulin production), showed increased pyruvate carboxylase (*PC*) and cytoplasmic malic enzyme 1 (*ME1*) expression consistent with active pyruvate cycling, a process utilized by mature β cells to sustain glucose-stimulated insulin secretion. In contrast, the "metastasis-like primary" (MLP) subtype showed greater monocarboxylate transporter 1 (*SLC16A1*/*MCT1*) and hexokinase 1 (*HK1*) expression, which is suppressed in mature β cells (5). Transcriptomic PanNET subtypes appear to have distinct metabolic preferences.

Transcriptomic PDA subtypes

PDAs have often been regarded as homogeneous due to the overwhelming prevalence of driver *KRAS* mutations. However, for the first time, we demonstrated that PDAs, like other cancers, can be classified into three gene expression subtypes using a 62-gene

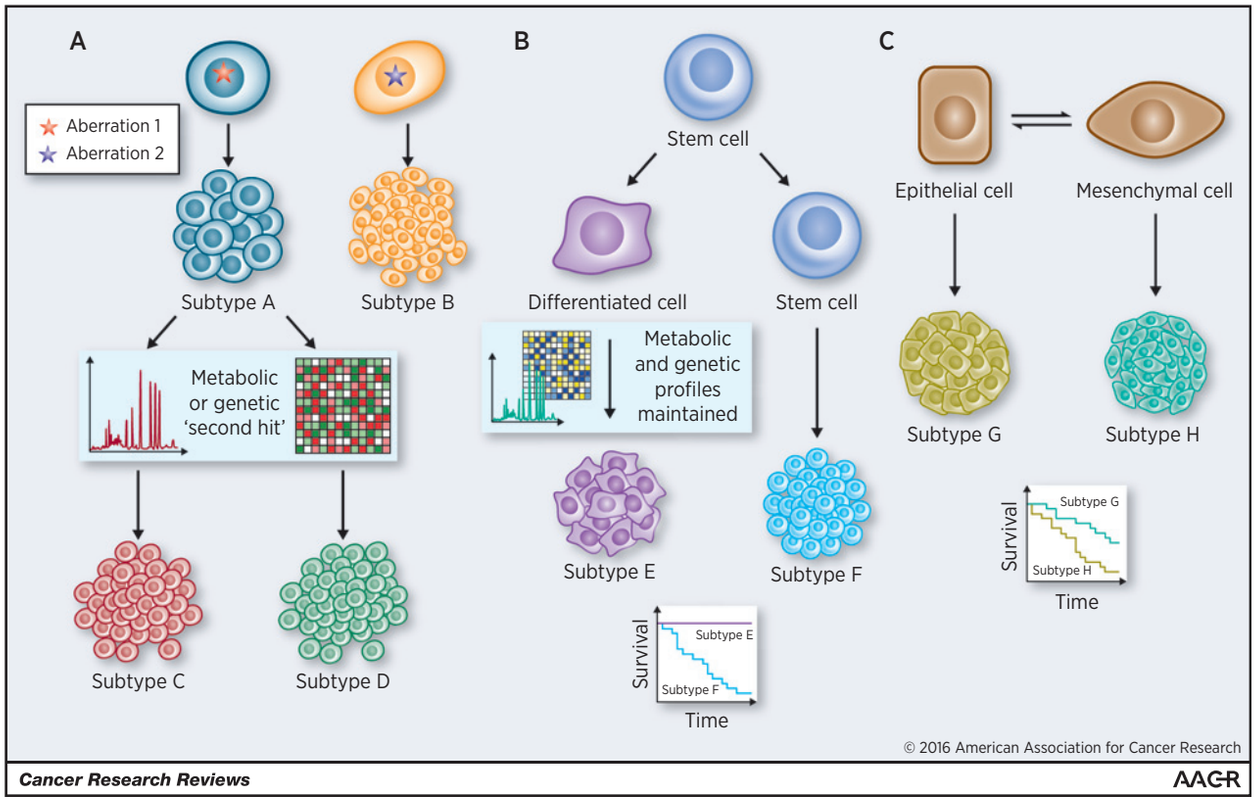

Figure 1.

Various origins of tumor heterogeneity. **A,** driver aberrations determine the characteristics of the ensuing tumor and, consequently, two driver aberrations (genetic or metabolic; aberration-1, e.g., *KRAS* mutation, shown as a red star inside the cell in the left panel, and a different one, aberration-2, e.g., an *EGFR* mutation, shown as a blue star inside the cell in the right panel) can result in distinct tumor subtypes (subtypes A or B). Subsequently, a second metabolic or genetic hit can determine tumor subtypes (left). A homogeneous tumor derived from a cell with a particular tumor-initiating driver aberration-1 can acquire a further metabolic or genetic hit for subsequent tumor progression. The nature of the second hit determines the associated characteristics of the progressing tumor subtype, leading to heterogeneity (subtypes C or D). **B,** heterogeneity can arise depending on the cell-of-origin. In certain cases, tumors that arise from well-differentiated cells (e.g., β cells) can result in a subtype (subtype E) that maintains both the metabolic and genetic profiles of the original cell-of-origin, and mostly have favorable prognosis (shown with a Kaplan–Meier curve). On the other hand, those tumors arising from stem/precursor cells with fewer markers of differentiation (subtype F) probably have fewer metabolic and genetic characteristics of their parental cells and have a poorer prognosis (shown with a Kaplan–Meier curve). **C,** EMT can lead to distinct tumor subtypes. Tumors originating from epithelial or mesenchymal cells can result in distinct subtypes (subtypes G and H, respectively) with different prognoses (shown with a Kaplan–Meier curve). EMT is shown with a reversible arrow.

signature (PDAssigner; ref. 3). One subtype, "classical PDAs (corroborated by Moffitt and colleagues; ref. 30)," is characterized by high adhesion-associated, ribosomal and epithelial gene expression, and elevated *GATA6* expression (3), which is essential for pancreatic development (31).

The second PDAssigner subtype shows high expression of tumor cell–derived exocrine genes and was hence named "exocrine-like" (3). We took particular care to enrich cancer cells by microdissection for PDA subtyping to identify cancer-specific subtypes, and further validated the presence of exocrine-like subtype by performing IHC to detect the cancer cell–specific expression of exocrine-like subtype proteins on gene expression subtype–matched PDA samples (3). The presence of exocrine-like subtype was validated by Noll and colleagues (32), by deriving matched exocrine-like PDA patient-derived xenograft tumors and cell lines. In addition, they have shown this subtype to be resistant to tyrosine kinase inhibitors and paclitaxel via a novel mechanism, suggesting the requirement for different personalized approach for this cancer subtype (32).

The third subtype, the "quasi-mesenchymal PDAs" (QM-PDA), exhibits high mesenchymal gene expression, representing a possible association with cancer-associated fibroblasts/stroma. Moreover, we clearly demonstrated increased glycolytic gene expression, including *MCT1*, hexokinase 2 (*HK2*) and glucose transporter 3 (*SLC2A3*/*GLUT3*) in QM-PDAs. Hence, QM-PDAs are a highly glycolytic PDA subtype with worse prognosis than the classical and exocrine-like subtypes (3). These subtypes were validated by independent studies involving patient PDA tumors (30, 32, 33) and cell lines (8), and by ourselves using GEM model–derived PDA cell lines (34). Although referred to as "basal-like" (based on a similarly-named breast cancer subtype) in Moffitt and colleagues (30), the QM-PDA subtype nomenclature was chosen to reflect the presence of both tumor and stromal genes in the signature (3). Interestingly, the PDA subtypes in Bailey and colleagues (33) almost entirely conformed to our PDAssigner subtypes, except for an additional "immunogenic" subtype, where their (i) "squamous" subtype represent our QM-PDA subtype, (ii) "pancreatic progenitor" represent our classical

subtype, and (iii) "aberrantly differentiated endocrine exocrine (ADEX)" represent our exocrine-like subtype (33). Conversely, there was lower concordance between the Moffitt and Bailey subtypes (33).

Importantly, classical and QM-PDA cell lines with different transcriptomes and metabolomes exhibit differential responses to two common therapies: classical PDA cell lines were more sensitive to erlotinib and QM-PDA lines to gemcitabine (3), despite increased mutant *KRAS* dependence in the classical subtype (3, 26). Patients with classical subtype tumors, therefore, may derive benefit from KRAS signaling related therapies, although this has yet to be realized clinically. However, these data provide clues as to why current clinical responses to erlotinib and gemcitabine in combination are heterogeneous in unselected PDA patients. Moffitt and colleagues have shown that basal-like (similar to QM-PDA) subtype patients were associated with better response to adjuvant therapy compared with those with classical subtype PDA (30), further suggesting personalized treatment options in this aggressive cancer type.

Metabolomic PDA subtypes

Complementary to the transcriptomic subtypes described above, metabolic profiling has also revealed three PDA subtypes (8): "glycolytic" PDAs (QM-PDAs), with elevated glycolysis and serine pathways, increased *MCT1* expression, and high glutamine incorporation into TCA cycle metabolites; "lipogenic" PDAs (classical PDAs), with lipid and electron transport chain metabolite enrichment and high lipogenesis gene expression, high oxygen consumption and mitochondrial content, and high glucose incorporation into TCA cycle metabolites; and "slow proliferating" PDAs low in amino acids and carbohydrates. These subtype-specific cell lines were also shown to have different responses to various metabolism-based inhibitors (8).

Putative Cell-of-Origin and Metabolic Phenotypes

An alternative hypothesis to distinct driver aberrations leading to different subtypes is that cancer cells with different cells-of-origin (27) or those that have undergone epithelial–mesenchymal transition (EMT) and having distinct molecular and/or metabolic profiles develop into distinct subtypes based on the cell's metabolic dependencies (Fig. 1B and C).

The cell-of-origin/phenotype of PanNETs

Combined transcriptomic and metabolic profiling can reveal patterns in phenotypes of cancer subtypes that are reminiscent of their normal counterpart cells, probably reflecting different cellular origin. In PanNET, IT tumors are clinically characterized as well differentiated, functional (secrete insulin), and low grade (have low Ki67-based proliferation index), which infrequently metastasize, and share gene expression and metabolism with mature islet β cells. Conversely, the proliferation rate in IT cells is comparatively higher than in β cells irrespective of the infrequent somatic mutations in tumors (5). Hence, ITs are likely to arise from more differentiated β cells. In this way, they are probably similar to the exocrine PDA subtype (3) and enterocyte and goblet-like/metabolic colorectal cancer subtypes (see below) (4), which all retain characteristics of their normal differentiated cells (all these are represented generally in Fig. 1B, left).

In contrast, MLP subtype tumors are poorly differentiated and nonfunctional (i.e. no hormones can be detected in the blood) and are associated with liver metastases and high tumor grades. This subtype possesses a typical pancreatic stem/precursor cells or immature β cell transcriptional signature and expresses genes associated with fibroblasts, stroma, stem cells, and hypoxia (see Fig. 1B).

The cell-of-origin/phenotype of colorectal tumors

We discovered five clinically pertinent colorectal cancer subtypes by mRNA profiling of 1,290 tumors ("stem-like", "transit amplifying", "enterocyte", "goblet-like", and "inflammatory" sutypes; ref. 4). Comparisons with known colon-crypt cell type gene signatures revealed likely cell-of-origin/phenotype candidates (see Fig. 1B). For example, the goblet-like and enterocyte subtype signatures were associated with those of the normal goblet and enterocyte cells (colon crypt top), while the stem-like subtype was associated with the crypt base, implicating these sites as the putative cell-of-origins for these subtypes. The stem-like subtype (with low differentiation marker expression) showed high stem cell, myoepithelial/mesenchymal, and stromal gene expression.

Although these profiles were subsequently independently confirmed (35), other studies have concluded that colorectal cancers can be divided into between three and six subtypes (36). Reconciliation of these subtypes has revealed that these classifications were in fact in broad agreement for four subtypes, with the remainder being further subdivisions of these "consensus molecular subtypes" (CMS; ref. 36). One of these four CMS subtypes, which maps to our differentiated goblet-like subtype, was dubbed the "metabolic" (or CMS3) subtype (36) due to its enrichment for several metabolic gene signatures, and was associated with high *KRAS* mutation frequency, whose influence on metabolism is discussed above (24, 25).

Epithelial and mesenchymal signatures in cancer subtypes

EMT is a phenotypic switch in which cancer cells convert to a more invasive and metastasis-capable (mesenchymal) state. As EMT is reversible it cannot simply be attributed to a genetic event, but instead is likely to represent a comprehensive reprogramming of the genetic, epigenetic, and metabolic profiles of the cell. This reprogramming is triggered by extracellular signaling, which results in genetic and metabolic adaptation to the microenvironment (refer to ref. 37).

Tumor stratification into subtypes can also reveal an epithelial or mesenchymal classification (Fig. 1C). In PDA, the classical subtype expresses high levels of epithelial genes including *CDH17* and *CEACAM6*, while QM-PDAs are enriched for the mesenchymal gene *TWIST1* (3, 8). In colorectal cancer, the stem-like (CMS4) subtype represents a mesenchymal phenotype, whereas goblet-like (CMS3) and enterocyte (subset of CMS2) colorectal cancers represent epithelial phenotypes (4). Similarly, in PanNETs, the IT subtype exhibits differentiated cell-based markers, whereas MLPs have mesenchymal signature along with increased glycolytic genes. Moreover, the mouse MLP subtype can be further subdivided into those that express low or high insulin gene/protein ("MLP Ins-lo" and "Ins-hi"): the Ins-lo subtype probably originates from pancreatic stem/islet precursor cells (Fig. 1B), while the Ins-hi tumors are likely to be the result of epithelial–mesenchymal transition from mature β cells or the progression of

β-cell–derived IT tumors based on their gene expression profiles (see Fig. 1C; ref. 5). This suggests that the epithelial and mesenchymal phenotypes in PanNET subtypes are products of their cells-of-origin and a consequence of subsequent reprogramming between the epithelial and mesenchymal states (5). Nevertheless, it would be interesting to examine the interactions of genetic, epigenetic, and metabolic factors that trigger EMT in these subtypes.

Concluding Remarks

Overall, there appear to be at least three broad divisions of the origins of tumor heterogeneity and subtypes based on metabolic, genetic, and/or molecular changes. The first are subtypes with initial tumorigenic driver genetic or metabolic aberration(s) that give rise to different tumor subtypes (Fig. 1A, left and right). Nonetheless, an initial aberration as a first hit in their normal counterparts is probably followed by the gain of additional secondary genetic or metabolic aberration(s) that further drive progression and affect patient prognosis (Fig. 1A, left). In the second main group of cancers, cell-of-origin determines the subtypes, with certain differentiated subtypes maintaining the transcriptomic and other important characteristics of their well-differentiated normal counterparts [ref. 38; e.g. pyruvate cycling (5)] and being addicted to more normal cellular energy metabolism. Most of these subtypes have favorable prognosis (Fig. 1B, left). Others, probably originating from stem/precursor cells, are likely to shift their energy metabolism toward glycolysis and other malignant metabolisms, and have a poorer prognosis (Fig. 1B, right). Finally, the malignant potential and metabolic reprograming are inversely correlated with differentiated cell-based marker expression, with epithelial and mesenchymal/stemness signatures resulting in different prognoses (Fig. 1C). Whether this context-specific metabolic reprogramming in different subtypes is triggered from the outset of tumor proliferation or occurs as a result of cellular adaptation still needs to be determined.

Nevertheless, it has recently become clear that tumor heterogeneity influences therapeutic efficacy in a variety of cancer types. Stratifying patients into groups that best respond to treatment based on the individual tumor's driver molecular aberrations has had clinical success. For example, tamoxifen and trastuzumab are two drugs that have subtype-specific benefits in patients with estrogen receptor and HER2-positive breast cancers, respectively (39, 40). Our recent work and that of others has indicated that these molecular indicators of drug/subtype specificity are also likely to exist in other cancers (3, 4, 6, 30, 35, 41, 42). However, whether metabolic changes, cell-of-origin, and EMT could be exploited for personalized/precise cancer therapies requires increased attention.

Disclosure of Potential Conflicts of Interest

A. Sadanandam has ownership interest (including patents) as a patent inventor for a a patent entitled "Colorectal cancer classification with different prognosis and personalized therapeutic responses" (patent number PCT/IB2013/060416). No potential conflicts of interest were disclosed by the other authors.

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References

1. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999;286:531–7.
2. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747–52.
3. Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med 2011;17:500–3.
4. Sadanandam A, Lyssiotis CA, Homicsko K, Collisson EA, Gibb WJ, Wullschleger S, et al. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. Nat Med 2013;19:619–25.
5. Sadanandam A, Wullschleger S, Lyssiotis CA, Grotzinger C, Barbi S, Bersani S, et al. A cross-species analysis in pancreatic neuroendocrine tumors reveals molecular subtypes with distinctive clinical, metastatic, developmental, and metabolic characteristics. Cancer Discov 2015;5:1296–313.
6. Verhaak RGW, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98–110.
7. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883–92.
8. Daemen A, Peterson D, Sahu N, McCord R, Du X, Liu B, et al. Metabolite profiling stratifies pancreatic ductal adenocarcinomas into subtypes with distinct sensitivities to metabolic inhibitors. Proc Natl Acad Sci U S A 2015;112:E4410–E7.
9. Dang CV.Links between metabolism and cancer. Genes Dev 2012;26:877–90.
10. Warburg O.On the origin of cancer cells. Science 1956;123:309–14.
11. Pasteur L.Mémoire sur la fermentation alcoolique. C R Seances Acad Sci 1857;45:1032–6.
12. Higgins LH, Withers HG, Garbens A, Love HD, Magnoni L, Hayward SW, et al. Hypoxia and the metabolic phenotype of prostate cancer cells. Biochimica et Biophysica Acta 2009;1787:1433–43.
13. Griguer C, Oliva C, Gillespie GY. Glucose metabolism heterogeneity in human and mouse malignant glioma cell lines. J Neurooncol 2005;74:123–33.
14. Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 2008;452:230–3.
15. Luo W, Semenza GL. Emerging roles of PKM2 in cell metabolism and cancer progression. Trends Endocrinol Metab 2012;23:560–6.
16. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009;462:739–44.
17. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med 2009;360:765–73.
18. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med 2009;361:1058–66.
19. Pollard PJ, Briere JJ, Alam NA, Barwell J, Barclay E, Wortham NC, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum Mol Genet 2005;14:2231–9.
20. Williamson SR, Eble JN, Amin MB, Gupta NS, Smith SC, Sholl LM, et al. Succinate dehydrogenase-deficient renal cell carcinoma: detailed characterization of 11 tumors defining a unique subtype of renal cell carcinoma. Mod Pathol 2015;28:80–94.
21. Hadoux J, Favier J, Scoazec JY, Leboulleux S, Ghuzlan AA, Caramella C, et al. SDHB mutations are associated with response to temozolomide in patients with metastatic pheochromocytoma or paraganglioma. Int J Cancer 2014;135:2711–20.

22. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, et al. Mutational landscape and significance across 12 major cancer types. Nature 2013;502:333–9.
23. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801–6.
24. Ying H, Kimmelman AC, Lyssiotis CA, Hua S, Chu GC, Fletcher-Sananikone E, et al. Oncogenic kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell 2012;149:656–70.
25. Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature 2013;496:101–5.
26. Singh A, Greninger P, Rhodes D, Koopman L, Violette S, Bardeesy N, et al. A gene expression signature associated with "K-Ras Addiction" reveals regulators of EMT and tumor cell survival. Cancer Cell 2009;15:489–500.
27. Visvader JE.Cells of origin in cancer. Nature 2011;469:314–22.
28. Kaelin WG, McKnight SL. Influence of metabolism on epigenetics and disease. Cell 2013;153:56–69.
29. Yun J, Johnson JL, Hanigan CL, Locasale JW. Interactions between epigenetics and metabolism in cancers. Front Oncol 2012;2:163.
30. Moffitt RA, Marayati R, Flate EL, Volmar KE, Loeza SGH, Hoadley KA, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet 2015;47:1168–78.
31. Decker K, Goldman DC, Grasch CL, Sussel L. Gata6 is an important regulator of mouse pancreas development. Dev Biol 2006;298:415–29.
32. Noll EM, Eisen C, Stenzinger A, Espinet E, Muckenhuber A, Klein C, et al. CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma. Nat Med 2016;22:278–87.
33. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016;531:47–52.
34. Kapoor A, Yao W, Ying H, Hua S, Liewen A, Wang Q, et al. Yap1 activation enables bypass of oncogenic KRAS addiction in pancreatic cancer. Cell 2014;158:185–97.
35. Medico E, Russo M, Picco G, Cancelliere C, Valtorta E, Corti G, et al. The molecular landscape of colorectal cancer cell lines unveils clinically actionable kinase targets. Nat commun 2015;6:7002.
36. Guinney J, Dienstmann R, Wang X, de Reyniès A, Schlicker A, Soneson C, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015;21:1350–6.
37. Li L, Li W. Epithelial-mesenchymal transition in human cancer: comprehensive reprogramming of metabolism, epigenetics, and differentiation. Pharmacol Ther 2015;150:33–46.
38. Hu J, Locasale JW, Bielas JH, O'Sullivan J, Sheahan K, Cantley LC, et al. Heterogeneity of tumor-induced gene expression changes in the human metabolic network. Nat Biotechnol 2013;31:522–9.
39. Jordan VC. Tamoxifen (ICI46,474) as a targeted therapy to treat and prevent breast cancer. Br J Pharmacol 2006;147:S269–76.
40. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
41. De Sousa E, Melo F, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LPMH, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. Nat Med 2013;19:614–8.
42. Heiser LM, Sadanandam A, Kuo W-L, Benz SC, Goldstein TC, Ng S, et al. Subtype and pathway specific responses to anticancer compounds in breast cancer. Proc Natl Acad Sci U S A 2012;109:2724–9.