Points Highlighted by Chad Macias, with supporting References

(All text from here on is Chads)

Glioblastoma very rarely metastasize. Although glioblastoma cells are found in blood vessel basement membrane surrounded by the extracellular matrix fibronectin they do not intravasate into the blood vessels or lymphatic system....."Glioblastoma is very rarely found outside the central nervous system. These data indicate that glioblastoma cells are confined to the central nervous system by an inability to pass through vital basement membrane"

https://www.ncbi.nlm.nih.gov/pubmed/7708148?dopt=Abstract

"Systemic metastasis of glioblastoma multiform (GBM) in the form of bulk tumor is rare. Brain tumor cells implanted outside the CNS formed tumors unless there was a significant difference between the immunotype of the implanted cells and host. These results support the hypothesis that the rarity of systemic GBM tumors lies in the presence of physical barriers and/or systemic hurdles that prevent their timely growth"

https://www.ncbi.nlm.nih.gov/pubmed/15936366

"Clinically, Glioblastoma metastasis rarely happen, we show that Glioblastoma lines can be highly invasive after ELM selection, but they still are not metastatic when implanted in the brain. The lack of extra cranial metastasis of the derivative GBM-M2 cell lines strongly suggests that rapid tumor growth or the unique CNS environment curtails the escape of tumor cells"

https://translational-medicine.biomedcentral.com/articles/10.1186/1479-5876-6-77

Establishing "External validity involves conducting replication studies that vary experimental conditions. Unforeseen factors that frustrate the transfer of cause-and-effect relationships from one system to another related system are “threats to external validity.” Researchers address threats to external validity by replicating treatment effects in multiple model systems" including GEMs and orthotropic and combination therapy models. Construct validity is what establishes Clinical Generalization. Responses in an inbred mouse, for example, may be particular to the strain, thus limiting generalizability to other mouse models or" human "patients.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3720257/#pmed.1001489.s002

"Therefore, to support the development of cancer therapeutics, there needs to be a series of models used, including GEMs and orthotropic and combination therapy models. Ideal primary tumor tissue xenografts result from patient-derived explants, established as models, at low passage numbers (<10 passes removed from patient). Furthermore, these cell lines are not grown in plastic or propagated as cell cultures. Establishing xenograft tumor models from patient-derived tumor tissue at low passage is believed to conserve original tumor characteristics such as heterogeneous histology, clinical biomolecular signature, malignant phenotypes and genotypes, tumor architecture and tumor vasculature.

The underlying limitations of tumor models also serve to reinforce the need for careful attention to design (applying correct models to the question), conduct (using multiple models) and interpretation (recognizing limitations and applying stringent criteria to outcomes) of efficacy studies for tumor modulation. Thus, while animal models can provide a form of “high throughput” in the selection of potential drug gable candidates, the use of these results to predict human clinical outcomes is premature. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3895289/

"The VM-M3 model of metastatic cancer is a novel murine model that closely mimics the natural progression of invasion and metastasis [7], [8]. The VM-M3 tumor arose spontaneously in the brain of a mouse of the VM/Dk inbred strain and expresses multiple growth characteristics of human glioblastoma multiforme with macrophage/microglial properties [7], [9]. When implanted subcutaneously, VM-M3 cells rapidly metastasize to all major organ systems, notably the liver, lung, kidney, spleen, brain, and bone marrow. Systemic metastasis has also been repeatedly documented in human glioblastoma multiforme (GBM), which has been linked to the macrophage/microglial characteristics of the tumor [9]. The tumor was adapted to cell culture and transfected with the firefly luciferase gene to allow for easy monitoring of tumor growth in vivo [10]. The VM-M3 model of metastatic cancer has a distinct advantage over other metastatic models because it spreads naturally in an immunocompetent host, mimicking the natural cancer microenvironment"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3673985/#pone.0065522-Skinner1

In "Spontaneous" metastasis assay(syngeneic or xenografts) cancer tissue or tumor cells are primarily implanted into the organ from which the cancer cells have been originally derived (orthotropic transplantation) or into a tissue of high vascularization and convenient anatomical location that is not representing the organ of origin (ectopic transplantation). Ectopic transplantation models mostly fail to mimic the appropriate microenvironment of the primary tumor and the corresponding metastatic dissemination to the relevant organs

http://www.sciencedirect.com/science/article/pii/S1574789113000331

"Syngeneic transplantation refers to the inoculation of murine cells into another mouse recipient of the same genetic background. Syngeneic cell lines are either derived from spontaneously developing tumors or from carcinogen-, transgene- or gene knockout-induced tumor. Syngeneic models are based on inbred mouse strains and therefore the host organism lacks the genetic heterogeneity of human patients (Fantozzi and Christofori, 2006; Khanna and Hunter, 2005)"

"The transplantable models primarily rely on the use of cancer cell lines which through continuous passaging in cell culture acquire notable as well as subtle changes that can significantly alter their properties (Fidler and Kripke, 1977). Moreover, although cell lines are heterogeneous, they may represent the expansion of a particular clone with high proliferative ability"

"For instance, a set of 70 “poor prognosis gene signature” has been identified for breast cancer metastasis (van 't Veer et al., 2002; van de Vijver et al., 2002). This signature encompasses genes regulating cell cycle, invasion, metastasis and angiogenesis and is a powerful predictor of disease outcome in young patients (van de Vijver et al., 2002). In an independent study, the gene expression profiles of metastases of multiple cancer types have been compared to unmatched primary adenocarcinomas, revealing a 128 gene metastasis signature that distinguishes primary from metastatic adenocarcinomas (Ramaswamy et al., 2003).

http://www.sciencedirect.com/science/article/pii/S1574789113000331

"2010: The First Human Cancer Treatment Vaccine

Food and Drug Administration (FDA) approves sipuleucel-T, a cancer treatment vaccine that is made using a patient's own immune system cells (dendritic cells), for the treatment of metastatic prostate cancer that no longer responds to hormonal therapy. It is the first (and so far only) human cancer treatment vaccine to be approved"

https://www.cancer.gov/research/progress/250-years-milestones

"Ketone bodies can replace glucose as the major source of brain energy when glucose becomes scarce. Although it is generally assumed that the liver supplies extrahepatic tissues with ketone bodies, recent evidence shows that astrocytes are also ketogenic cells. Moreover, the partitioning of fatty acids between ketogenesis and ceramide synthesis de novo might control the survival/death decision of neural cells"

https://www.ncbi.nlm.nih.gov/pubmed/11295573/

"Like hepatocytes, astrocytes possess an enzymatic equipment capable of synthesizing large amounts of ketone bodies. In addition, both astrocytes and hepatocytes exhibit a preference for

fatty acids, compared with glucose, as their primary metabolic fuel. Recent determination of flux control coefficients of the enzymes involved in ketogenesis shows that CPT-I catalyzes the pace-setting step of ketogenesis from long-chain fatty acids in both hepatocytes and astrocytes. Data in the present report showing that changes in the rate of ketogenesis upon activation of AMPK rely on parallel changes in CPT-I activity are in agreement with that notion. Likewise, the stimulation of ketogenesis by cyclic AMP-raising agents and ceramide-generating compounds was shown to be independent of changes in mitochondrial HMG-CoA synthase activity"

http://onlinelibrary.wiley.com/doi/10.1046/j.1471-4159.1999.731674.x/full

"Here, we directly evaluate whether the end-products of aerobic glycolysis (3-hydroxy-butyrate and L-lactate) can stimulate tumor growth and metastasis. We show that administration of 3-hydroxy-butyrate (a ketone body) increases tumor growth by ~2.5-fold, without any measurable increases in tumor vascularization/angiogenesis. Both 3-hydroxy-butyrate and L-lactate functioned as chemo-attractants, stimulating the migration of epithelial cancer cells. Lastly, our findings may explain why diabetic patients have an increased incidence of cancer, due to increased ketone production, and a tendency towards autophagy/mitophagy in their adipose tissue"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3047616/

"Catabolic fibroblasts, with mitochondrial dysfunction, produce ketone bodies in the tumor stroma. Then, these ketone bodies are re-utilized by adjacent cancer cells, which process these ketone bodies as mitochondrial fuels for oxidative phosphorylation (OXPHOS), to drive anabolic tumor growth. Our results directly show that ketone body production and re-utilization can drive increased tumor growth and metastasis. Thus, ketone bodies behave as onco-metabolites, and we directly show that the enzymes HMGCS2, ACAT1/2 and OXCT1/2 are bonafide metabolic oncogenes. As such, the enzymes associated with ketone body production and re-utilization should be considered as new "draggable" targets for anticancer therapy"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3507492/?log%24=activity

"We showed here that both ketones and lactate promote the growth of embryonic stem (ES) cells. Consistent with these findings, a recent study showed that ES cells preferentially use mitochondrial oxidative metabolism, and that their dependence on mitochondria decreases as they undergo differentiation. This fits well with the idea that ES cells use lactate and ketones as fuel for the TCA cycle and oxidative mitochondrial metabolism, thereby stimulating stem cell growth. In accordance with our assertion that cancer cells use mitochondrial oxidative phosphorylation for energy production, metformin treatment prevents and/or inhibits tumor formation both in diabetic patients and in mouse animal models. Moreover, metformin also kills “cancer stem cells”. We see that these high-energy metabolites induce a “stem-like” transcriptional profile that is specifically associated with tumor recurrence, metastasis and poor clinical outcome. Finally, it is quite ironic that such a promising anti-cancer drug (metformin) exerts its therapeutic effects by inducing a type of

metabolism (aerobic glycolysis) that has been proposed to be the “root-cause” of cancer for the last 85 years. Thus, induction of aerobic glycolysis in cancer cells may not be the “cause of cancer,” but rather it may be the “cure for cancer.”

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3117136/

"L-lactate derived from glycolytic fibroblasts is transferred to cancer cells and is used to generate energy via oxidative mitochondrial metabolism (OXPHOS). Similarly, ketone bodies and glutamine derived from host cell catabolism can also fuel the mitochondrial activity of adjacent epithelial cancer cells. We have termed this new form of parasitic cancer metabolism the “reverse Warburg effect” (since increased glycolysis occurs in fibroblasts rather than tumor cells) or the “auotphagic tumor storm model of cancer” (since tumor cells induce autophagy and mitophagy in adjacent fibroblasts)"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3272257/

"Induction of oxidative stress and autophagy/mitophagy in the tumor stromal compartment provides a means by which epithelial cancer cells can directly "feed off" of stromal-derived essential nutrients, chemical building blocks (amino acids, nucleotides), and energy-rich metabolites glutamine, pyruvate, ketones/BHB, driving tumor progression and metastasis. Essentially, aggressive cancer cells are "eating" the cancer-associated fibroblasts via autophagy/mitophagy in the tumor micro-environment. Lastly, we discuss that this "Autophagic Tumor Stroma Model of Cancer Metabolism" provides a viable solution to the "Autophagy Paradox" in cancer etiology and chemo-therapy"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3047615/

"As results, HFD enhanced melanoma burden in bone by increasing tumor area and osteoclast numbers. This process was associated with higher numbers of bone marrow adipocytes expressing IL-6 in direct vicinity to tumor cells. Metabolic stress by HFD promotes melanoma growth in the bone marrow by an increase in bone marrow adipocytes and IL-6-JAK2-osteopontin mediated activation of tumor cells and osteoclast differentiation"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5042005/

"Tumor-associated fibroblasts release high-energy metabolites (L-lactate and ketones) and chemical building blocks (nucleotides, fatty acids and amino acids, such as glutamine). These catabolites stimulate mitochondrial biogenesis, OXPHOS and autophagy-resistance in epithelial cancer cells, and protect cancer cells against chemotherapy-induced apoptosis. In summary, we believe that a critical biological function of the tumor storm is to produce L-lactate and other high-energy catabolites

(such as ketones and glutamine) to “fuel” oxidative mitochondrial metabolism (OXPHOS) in adjacent epithelial cancer cells"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3335917/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3272287/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3335944/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3335942/

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3272288/

"Intra-abdominal tumors, such as ovarian cancer, have a clear predilection for metastasis to the momentum, an organ primarily composed of adipocytes. Adipocyte-ovarian cancer cell coculture led to the direct transfer of lipids from adipocytes to ovarian cancer cells and promoted in vitro and in vivo tumor growth. Furthermore, coculture induced lipolysis in adipocytes and β-oxidation in cancer cells, suggesting adipocytes act as an energy source for the cancer cells. A protein array identified up regulation of fatty acid-binding protein 4 (FABP4, also known as aP2) in mental metastases as compared to primary ovarian tumors, and FABP4 expression was detected in ovarian cancer cells at the adipocyte-tumor cell interface. These data indicate adipocytes provide fatty acids for rapid tumor growth, identifying lipid metabolism and transport as new targets for the treatment of cancers where adipocytes are a major component of the microenvironment.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4157349/

"Thus, we believe that one of the major functions of the tumor stroma is to produce lactate and other high-energy nutrients (such as ketones and glutamine) to “fuel” oxidative mitochondrial metabolism in epithelial cancer cells. In accordance with this hypothesis, the intra-peritoneal (i.p.) injection of mouse xenografts with ketones or lactate is sufficient to significantly increase tumor growth (~2.5-fold) and metastasis (~10-fold), respectively. Interestingly, these gene signatures indicate that lactate and ketones increase the “stemness” of cancer cells, based on an analysis of their transcriptional profiles. Most importantly, however, these lactate- and ketone-induced gene signatures can also be used to predict clinical outcome in human breast cancer patients, and are associated with recurrence, metastasis and significant decreases in overall survival. Thus, we have suggested that lactate and ketones both function as tumor promoters, via their ability to drive increased oxidative mitochondrial metabolism in cancer cells"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142461/

"Prostate and breast cancer cells in particular have been specifically shown to employ fatty acid oxidation as a metabolic strategy"

http://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-11-56

http://mct.aacrjournals.org/content/13/10/2361?ijkey=66730f0c2ad6dcfa5da193301c705d4edae1f8ad&keytype2=tf_ipsecsha

"Gliomas have been thought to rely upon glycolysis for energy production, yet recent results from human NMR spectroscopy studies suggest that glucose contributes to <50% of acetyl-CoA production in gliomas. We observed the presence of enzymes required for fatty acid oxidation within human glioma tissues. In addition, we demonstrated that this metabolic pathway is a major contributor to aerobic respiration in primary-cultured cells isolated from human glioma and grown under serum-free conditions. Moreover, inhibiting fatty acid oxidation reduces proliferative activity in these primary-cultured cells and prolongs survival in a syngeneic mouse model of malignant glioma. Fatty acid oxidation enzymes are present and active within glioma tissues. Targeting this metabolic pathway reduces energy production and cellular proliferation in glioma cells"

http://neuro-oncology.oxfordjournals.org/content/early/2016/06/29/neuonc.now128.full

As far as brain cancers such as Glioblastoma Grabacka et al. suggested "Malignant cells derived from brain tissue (neuroblastoma, glioblastomas multiform astrocytomas, and schwannomas) either do not express SCOT and ACAT or express these enzymes at very low levels, making them incapable of using ketone body oxidation for ATP production. In fact, neuroblastoma and glioblastoma cells are only able to utilize ketone bodies as substrates for lipid synthesis"

http://journal.frontiersin.org/article/10.3389/fendo.2016.00005/full

But Schwarz et al. using a ketogenic diet as a mono therapy in patients with Glioblastoma observed the expression of two critical mitochondrial ketolytic enzymes, advancement in tumor growth and creation of new lesions during a 12 week intervention. "It has been proposed that energy-restricted ketogenic diets (ERKD) might serve as a metabolic treatment to improve survival of primary brain cancer patients. Subjects were trained by an experienced registered dietitian (RD) to assure competency for adherence to the ERKD protocol. The energy-restricted ketogenic diets protocol was to be administered for 12 weeks as medically appropriate. The patients who enrolled in our ERKD pilot study were monitored with twice daily measurements of blood glucose and ketones and daily weights. However, both patients showed tumor progression while on the energy-restricted ketogenic diet therapy. Immunohistochemistry reactions showed that their tumors had tissue expression of at least one of the two critical mitochondrial ketolytic enzymes succinyl CoA: 3-oxoacid CoA transferase, beta-3-hydroxybutyrate dehydrogenase 1. These data suggest that some of the malignant cells in these patients' cancers could metabolize ketones and derive energy for subsequent growth.

https://cancerandmetabolism.biomedcentral.com/articles/10.1186/s40170-015-0129-1

Many researchers suggest Glioma's (brain cancer) cannot metabolize ketones and would therefore "starve" the tumor if placed on a ketogenic diet, Grabacka et al. documented this "Malignant cells

derived from brain tissue (neuroblastoma, glioblastomas multiform astrocytomas, and schwannomas) either do not express SCOT and ACAT or express these enzymes at very low levels, making them incapable of using ketone body oxidation for ATP production. In fact, neuroblastoma and glioblastoma cells are only able to utilize ketone bodies as substrates for lipid synthesis"

http://journal.frontiersin.org/article/10.3389/fendo.2016.00005/full

But Schwarz et al. using a ketogenic diet as a mono therapy in patients with Glioblastoma observed the expression of two critical mitochondrial ketolytic enzymes, advancement in tumor growth and creation of new lesions during a 12 week intervention. "It has been proposed that energy-restricted ketogenic diets (ERKD) might serve as a metabolic treatment to improve survival of primary brain cancer patients. Subjects were trained by an experienced registered dietitian (RD) to assure competency for adherence to the ERKD protocol. The energy-restricted ketogenic diets protocol was to be administered for 12 weeks as medically appropriate. The patients who enrolled in our ERKD pilot study were monitored with twice daily measurements of blood glucose and ketones and daily weights. However, both patients showed tumor progression while on the energy-restricted ketogenic diet therapy. Immunohistochemistry reactions showed that their tumors had tissue expression of at least one of the two critical mitochondrial ketolytic enzymes succinyl CoA: 3-oxoacid CoA transferase, beta-3-hydroxybutyrate dehydrogenase 1. These data suggest that some of the malignant cells in these patients' cancers could metabolize ketones and derive energy for subsequent growth.

https://cancerandmetabolism.biomedcentral.com/articles/10.1186/s40170-015-0129-1

"The capability of performing ketolysis is a sign of metabolic flexibility of cancer cells and gives them a straightforward growth advantage. Breast cancer cells retain the capacity to perform oxidative metabolism at a high rate and take advantage of ketolysis to support their growth and progression. In breast and prostate carcinomas, expression of ketolytic enzymes is even regarded as a prognostic marker that is associated with aggressive phenotypes"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5187893/

"Prostate cancer is the most common malignancy and the second leading cause of cancer-related deaths in men. Over time, patients develop tumors that are androgen-independent and ultimately fatal. The mechanisms that cause this transition remain largely unknown. Our top candidate, HMGCS2, an enzyme involved in ketogenesis, was found to be 9-fold elevated. Taken together, our results indicate that enzymes of the ketogenic pathway are up-regulated in high-grade prostate cancer and could serve as potential tissue biomarkers for the diagnosis or prognosis of high-grade disease.

In order for cancer cells to proliferate and survive, they must meet their high energy demand for carrying out integral cellular processes. Cell growth, proliferation, and migration require large amounts of energy in the form of ATP, and by using alternative energy-producing pathways, cancerous cells gain a survival advantage. The ketogenic pathway is such an alternative energy-producing pathway, primarily responsible for the production of ketone bodies from fatty acids via the breakdown of acetyl-CoA, a key molecule formed during fatty acid metabolism. Acetyl-CoA, under normal high-glucose conditions, is oxidized, resulting in the formation of the high-energy molecules NADH and FADH2 in the citric acid cycle. However, when levels of acetyl-CoA are higher than required for the citric acid cycle, it is used for the biosynthesis of ketone bodies through the aid of five cellular enzymes. From our initial proteomics data, we identified all five of these enzymes as up-regulated and we also found an increase in β-hydroxybutyrate, the most common ketone body, in the secretome of these cells. These observations suggest that the ketogenesis pathway might be an alternate energy-producing mechanism through which prostate cancer cells gain a survival advantage allowing them to become increasingly aggressive and gain androgen-independent properties"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3675816/

"Results suggest that many of these tumors have alterations in mitochondrial metabolism. On the other hand, the positive expression of ACAT1, also a mitochondrial enzyme, in most tumors suggests that the observed decreases of OXCT1 and BDH1 do not necessarily reflect a complete loss or absence of mitochondria enzymes in these tumors. Nevertheless, our results showing that GBMs from different patients have different expression of these enzymes are consistent with previous molecular genetic studies showing that these are genetically heterogeneous tumors. Our results are also consistent with a recent study showing variable but positive expression of the ketone body metabolizing enzymes in several human glioma cell lines. Our results suggest that the differential expression of these enzymes could serve as potentially useful biomarkers to select human glioma patients who may or may not respond optimally to KD"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3707813/

"Ketogenic-diet (KD) was suggested as a therapeutic option for malignant brain cancer. This study aimed to detect metabolic brain changes in patients with malignant brain gliomas on KD using proton magnetic-resonance-spectroscopy (1H-MRS). Fifty MR scans were performed in four patients with recurrent glioblastoma (GB) treated with KD in addition to bevacizumab. High adherence to KD was obtained, in these patients ketone bodies, Acetone and Acetoacetate were detected in four MR spectra—three within the NAWM and one in the lesion area −4 and 25 months following initiation of the diet. 1H-MRS was able to detect ketone-bodies in patients with brain tumors who adhered to KD"

https://link.springer.com/article/10.1007/s11060-016-2364-x

"Even in the presence of oxygen, malignant cells often highly depend on glycolysis for energy generation. To investigate whether a ketogenic diet might selectively impair energy metabolism in tumor cells In vivo a non-calorie-restricted ketogenic diet was examined in an orthotopic xenograft glioma mouse model. In vivo, the ketogenic diet led to a robust increase of blood 3-hydroxybutyrate, but did not alter blood glucose levels or improve survival"

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3199865/

"These results suggest that the utilization of 3-hydroxybutyrate as an energy source by astrocytes is regulated in part by carrier-mediated transport and that the uptake system is different from the lactate transport system"

https://www.ncbi.nlm.nih.gov/m/pubmed/7891839/

"Neuron, astrocyte, and oligodendrocyte cultures were examined for their utilization of glucose, ketone bodies, and free fatty acids by oxidative processes. All three cell populations readily utilized the ketone bodies for oxidative metabolism at rates 7-9 times greater than they utilized glucose"

https://www.ncbi.nlm.nih.gov/m/pubmed/3481403/

"Ketone bodies serve as sources for energy and as precursors for lipid synthesis in developing brain. Using purified populations of neurons and astrocytes and purified oligodendroglia from bovine brain, the activities for the three enzymes involved in ketone body metabolism were evaluated. Surprisingly, astrocytes had the highest levels of activity for both 3-ketoacid-CoA transferase and acetoacetyl-CoA thiolase; these activites showed dramatic changes during development. Nonetheless, neurons, astrocytes and oligodendroglia are all quite capable of using ketone bodies as metabolic fuels"

https://www.ncbi.nlm.nih.gov/m/pubmed/20501089/