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Review Article

Metabolic Killing of Mitosis Addicted Cancer Cells by Targeting Aerobic Glycolysis: A New Achilles Heel of Cancer

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Abstract

The term "Achilles Heel" has often been used in relation to potentially vulnerable target molecules for anticancer drugs within cancer cells. Its generalized application to an increasing range of diverse possibilities, however, detracts from the uniquely valuable way it describes the wild-type aerobic glycolysis target which persists within the immortal cancer cell phenotype. Here it is proposed that the persistence of the PKM2 isoform of the normal non mutant pyruvate kinase gene which induces aerobic glycolysis in a cancer cell whose phenotype has otherwise been immortalized by oncogenic somatic mutation provides a close analogous comparison with the immortal body of Achilles and his mortal vulnerable heel. Exploitation of the mechanism believed to explain the way aerobic glycolysis helps avoid oncogene induced apoptosis has led to the development of ONKONEK, a first in class therapeutic agent employing the new therapeutic paradigm of selective cancer cell limotherapy (SCCL) which starves cancer cells rather than attacking their cell division and is not expected to be restricted by the limitations of current chemotherapy.

The new therapeutic paradigm of selective cancer cell limotherapy (SCCL) starves cancer and, in particular, down-regulates PKM2 activity during cytokinesis in mitosis addicted cancer cells. Selective cancer cell limotherapy, by sparing normal cells provides a potential global cancer treatment which could potentially be delivered repeatedly until every cancer cell is destroyed.

Keywords: Aerobic glycolysis, Cancer, CDK4, Limotherapy, PKM2

Achilles Heel

The mythological story tells how the goddess Thetis, a sea nymph tried to immortalize her son, whose father Peleus was a mortal, by dipping him in the river Styx. Because she grasped him by the heel, the water did not touch that part of his body which remained a vulnerable target for the fatal arrow later shot by Paris during the Siege of Troy. The concept of the presence of a vulnerable "Achilles Heel" in an immortal cancer cell has been widely employed in the literature and has grown to describe a multiplicity of unrelated potentially vulnerable target sites in cancer that might be treated without damage to normal tissues [1–5]. In some cases, even multiple "heels" have been included in this encompassing title [6]. No clinically effective anticancer drug has yet, however, resulted from this paradigm. Most chemotherapy is restricted by cumulative toxicity because it permanently damages normal as well as cancer cells. Chemotherapy can only therefore generally be administered for a limited number of courses and cannot achieve enough repeated treatments to completely eradicate the majority of cancers.

Here it is proposed that the persistence of the PKM2 isoform of the normal non mutant pyruvate kinase gene which induces aerobic glycolysis in a cancer cell provides the closest analogous comparison with the immortal body of Achilles and his mortal vulnerable heel.

Aerobic Glycolysis

Heinrich Friederich Warburg was the first to identify disordered carbohydrate metabolism as a significant characteristic of

cancer cells nearly a century ago. Since that time there has been a progressive interest in what subsequently came to be described as aerobic glycolysis [7], where cancer cells use only the glycolytic part of the carbohydrate catabolism pathway without the contribution of mitochondrial oxidative phosphorylation despite the abundant availability of oxygen. The mechanism underlying aerobic glycolysis is now known to be due to the presence within cancer cells of the PKM2 isoform of the non-mutated pyruvate kinase gene brought about by a switch of expression of exon 9 to exon 10, rather than the PKM1 isoform characteristic of normal adult mammalian cells [8]. Aerobic glycolysis is ubiquitously found in all human cancer cells in which it has been sought including myeloma, pediatric and pancreatic tumors [9–11].

The idea that normal cellular processes might play an important role in the etiology of cancer has rarely been considered [12]. The suggestion that a cause of cancer might lie in carbohydrate metabolism as suggested by Warburg did not meet with general scientific approval for many years and the ubiquitous presence of aerobic glycolysis throughout cancer still resists a convincing explanation [13]. The discovery of aerobic glycolysis was long considered to be a metabolic quirk of transformed cells, but recent studies in stem cells have challenged this model revealing that many normal stem cell populations also exhibit aerobic glycolysis [14,15]. Selectively killing cancer cells cannot therefore only rely on targeting aerobic glycolysis but also needs to operate in the context of the fundamental difference in mitotic cell division between cancer cells and normal stem cells, which is itself dependent on the key hallmarks of cancer: self-sufficiency in growth signals and limitless replicative potential [16]. These together account for the continuously repeated cycles of relentless cell division characteristic of all cancers, here termed mitotic addiction.

When Does Aerobic Glycolysis Arise in a Cancer Cell?

Recently it has been proposed that the reason for the presence of aerobic glycolysis in a cancer cell is because it is a preneoplastic change which is already present in the normal cell before it became transformed into a cancer cell by an oncogenic mutation [17,18].

Rather than being a consequence of malignant transformation, as previously thought, aerobic glycolysis in fact precedes malignant transformation (**Figure1**). This proposition is supported by reports that markers of aerobic glycolysis have recently been found in premalignant tissues [19,20].

The persistence of the PKM2 isoform switch through succeeding generations of the newly created cancer provides an explanation for its presence in all cancer cells. **Figure 1** compares the canonical somatic mutation theory of cancer (**A**) and the Achilles Heel modification (**B**), adoption of which implies that the cancer cell should be considered a hybrid mortal/immortal cell like the mythical Achilles with a mortal aerobic glycolysis "heel" in an otherwise immortal "body".
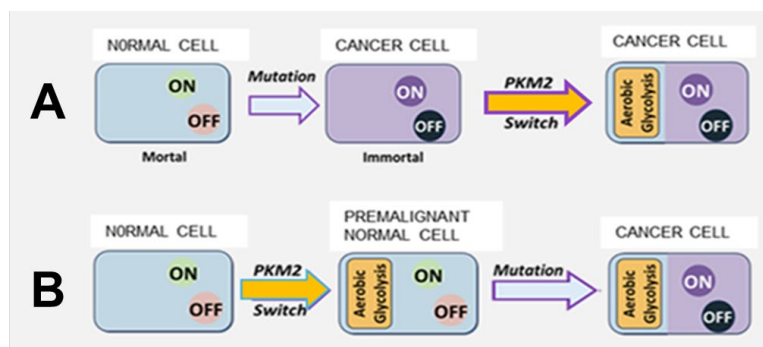

Figure 1. **Somatic mutation and aerobic glycolysis co-operate in oncogenesis. A.** Depiction of the widely accepted Somatic Mutation theory of cancer in which mutations of 'ON' switches controlling normal cell division create dominant oncogenes which drive increased cell division and mutations which inactivate "OFF" switches (suppressor genes) remove the brakes on cell division. This results in a normal cell becoming an immortalized cancer cell wholly driven by mutation. **B.** The new Oncogene/Normal Gene Interaction hypothesis of cancer which incorporates an intermediate stage with a preneoplastic switch to aerobic glycolysis. Counterintuitively, the interaction of two wild-type gene properties, normal PKM2, responsible for aerobic glycolysis and a non-kinase function of Cdk4 are required to facilitate malignant transformation without apoptosis by a somatic mutation in a cell division driver gene. The initial premalignant aerobic glycolysis phenotype which allows the nascent cancer cell to avoid apoptosis persists through the later generations of the cancer where it also has a role as the energy provider for cytokinesis.

The widely held earlier belief that each of the many different types of cancer cell, caused by different types of oncogenic somatic mutation arising in a wide range of different normal cells, suddenly "decides" to effect an identical change in the isoform expression of the same single normal gene, pyruvate kinase, (the suggested reasons being different from case to case) in every cancer cell, seems an unlikely explanation for the ubiquitous presence of aerobic glycolysis throughout cancer.

Oncogenic Mutations Require Normal CDK4 and PKM2 Interaction in Order to Achieve Immortalization with Avoidance of Apoptosis

In advanced cancers reasons provided for escape from apoptosis by cancer cells have been suggested to be increasing or decreasing expression of anti- or proapoptotic genes, respectively or stabilizing or de-stabilizing anti- or pro-apoptotic proteins, respectively [21]. At the moment of initial malignant transformation of a normal cell to a cancer cell a simpler method of avoiding apoptosis would be needed. Apoptosis is an energy dependent process. ATP availability determines whether necrosis or apoptosis is a cell's final fate [22]. It has now been proposed that at the moment of malignant transformation, in addition to activating relentless cell division through the canonical Cdk4/CyclinD-pRb-E2F pathway, In the G1 phase of the cell cycle (**Figure 4**) Cdk4 levels rise and interact with tetrameric PKM2 causing a transient lowering of intracellular ATP that is sufficient to prevent apoptosis but not sufficiently low to cause necrosis.

Pyruvate Kinase M2

The aerobic glycolysis phenotype in cancer cells is a consequence of the isotypic switch in expression of the pyruvate kinase gene to express the PKM2 isotype by expression of exon 10 rather than the PKM1 isotype expressed by exon 9. This results in a different amino acid sequence between amino acid sites 379–411 [23]. PKM2 can exist as a dimer with multiple interaction sites and functions or may form a tetramer which controls the transformation from phosphoenolpyruvate to pyruvate with the release of one molecule of ATP [24] (**Figure 2A**). The pyruvate is then converted into lactic acid by the enzyme lactate dehydrogenase.
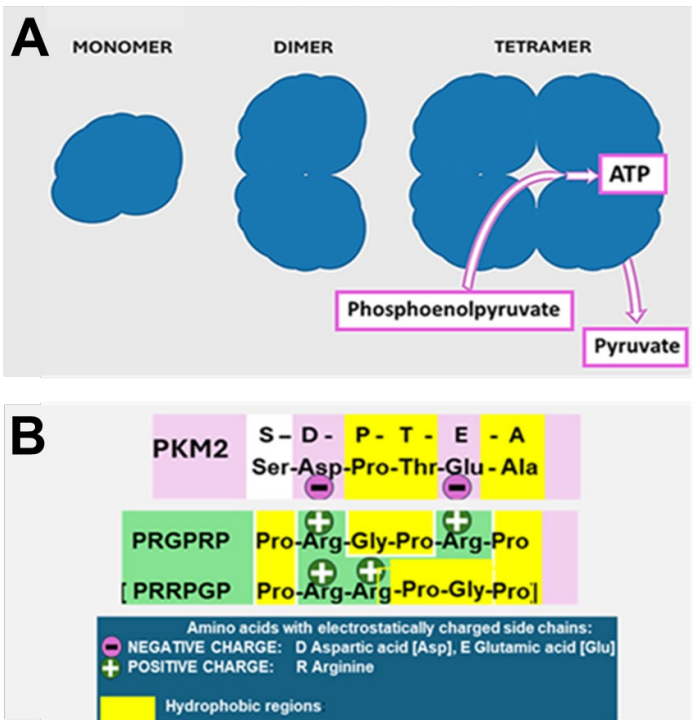

Figure 2. Pyruvate kinase M2. A. Gross molecular structure of PKM2 dimers and tetramers. Monomeric PKM2 has no reported functionality. Dimeric PKM2 has multiple molecular interactions, predominantly nuclear, engaged in signaling to and responding to intracellular molecules [17]. The metabolic function of PKM2 is limited to the tetrameric form located in the cytoplasm, which is modulated allosterically by fructose 1,6 biphosphate producing ATP by conversion of phospho-enol pyruvate to pyruvate and inhibited by the hexapeptide PRGPRP found in a C'-terminal external loop of Cdk4. **B. Proposed amino-acid sequence of PKM2 interaction site with PRGPRP.** A visual search of the amino acid sequences of key proteins involved in the glycolytic pathway revealed only one potential interacting site for PRGPRP which matched the strongly cationic twin arginines to ionically attractive glutamic and aspartic acids in a hydrophobic background. The potentially interactive site, SDPTEA lay at the C' end of the tetrameric junctional region. The figure shows potential alignment of PRGPRP but not PRRPGP.

Dimeric PKM2

Whereas PKM2 exerts its metabolic glycolytic function in the cytosol predominantly in its tetrameric form, the PKM2-specific exon 10 encodes a nuclear localization signal facilitating dimeric PKM2 import into the nucleus [24]. Nuclear translocation requires post-translational changes such as activation of ERK1/2 by EGRF signaling which causes ERK1/2 to bind to Ileu429/Leu431 and phosphorylate PKM2 at Ser37 [25]. The dimer transfers into the nucleus in the G1 phase of the cell cycle where it becomes involved in many signaling interactions including binding to phosphorylated-Tyr333 of β-catenin and phosphorylation of PKM2 at Thr454 which facilitates the transcriptional co-activation of HIF-1α and β-catenin [26] ultimately resulting in upregulation of cyclin D [27]. The dimeric form of PKM2 not only passively avoids participating in glycolysis but using PEP as a phosphate donor acts as a signaling molecule which, catalyzes tyrosine phosphorylation of STAT3 [28].

Tetrameric PKM2

The tetrameric form of PKM2 (**Figures 2A** and **2B**) is responsible for control of the rate-limiting step in glycolysis that shifts glucose metabolism from the normal respiratory chain to lactate production [29]. In contrast to the multiple signaling interactions of its dimeric form, the role of tetrameric of PKM2 is the generation of ATP derived from the transformation of phosphoenolpyruvate to pyruvate. Unlike the complex picture of modulation and activation of the PKM2 dimer, less information is available about the control of PKM2 tetramerization and its energy producing role except that PKM2 activators such as fructose-1-6-biphosphate (FBP) promote allosteric changes which influence active tetramer formation [30]. This activity is abrogated by a site directed mutation of PKM2 at Cys-423 within the tetramerization zone. In summary, PKM2 dimer can be regarded as an active protein kinase signaling moiety and PKM2 tetramer is an active pyruvate kinase providing intracellular energy in the form of ATP [31].

An Arginine Rich Amino Acid Sequence from the C'-terminal Non-kinase Region of CDK4 Downregulates ATP

An isolated PRGPRP construct in a cyclic amphiphilic cassette was shown to cause a fall in intracellular ATP and the global death of a wide range of human cancer cell lines by necrosis [19,25]. Normal diploid human keratinocytes exposed to PRGPRP underwent cell cycle arrest but did not die. The fall in cancer cell ATP was accompanied by inhibition of lactate dehydrogenase (a downstream indicator of PKM2 activity). Time lapse photomicrography [**in appendix**] shows that human lung cancer cells went through only a single division and then died as a result of exposure to THR 53, a construct containing the PRGPRP warhead in a cyclic amphiphilic vector of Ala/Leu/Lys repeats. The depletion of energy by PRGPRP appears to be critical for cancer cells to survive cell division. A modification of the PRGPRP warhead creating an increase in lipophilicity by the substitution of the warhead prolines by unnatural amino acids and incorporation into a ArgArg/TrpTrp repeat cassette to yield a previously described potential therapeutic agent, HILR-056 [19] has been renamed ONKONEK and has recently entered preclinical animal trials.

THR53 Docks at the End of the PKM2 Tetramerization Interface

THR53, a cyclic amphiphilic derivative of the Cdk4 C'-terminal hexapeptide Pro-Arg-Gly-Pro-Arg-Pro [32], was found to inhibit lactate dehydrogenase at dose which caused a fall in ATP accompanied by selective cancer cell necrosis. A comparison of the linear amino acid sequences of PKM1 and PKM2 was therefore undertaken in a search for a putative binding site for PRGPRP [17]. This revealed a Ser-Asp-Pro-Thr-Glu-Ala site unique to PKM2 lying at one end of the tetramerization interface, as a potential ionic interactive site for PRGPRP. It is proposed this interaction allosterically down-modulates ATP production.

Structural chemical modelling by computer modelling [previously unpublished data] is consistent with the possibility of a PRGPRP warhead carried in an Ala-Lys-Leu-repeat amphiphilic cassette (THR53) docking at the predicted SDPTEA site on the A, B, C, and D subsites of tetrameric PKM2 (**Figure 3**). **Figure 3** depicts the simulated plausible interaction site for PRGPRP in a THR 53 cassette lying in the A subunit of tetrameric PKM2. This interaction is repeated on subunits B, C, and D of the symmetrical molecule. The interaction (depicted in detail in **Figure 2B**) is ionic not covalent and is thus its maintenance is dependent on the continuous presence of PRGPRP and leaves no permanent molecular damage. Conventional chemotherapy irreversibly damages normal as well as cancer tissues which permits it to only be administered for a limited number of treatments which are, in general, insufficient to eradicate the majority of cancers. Because SCCL agents are likely to have a transient non-damaging effect on normal cells they can potentially be given repeatedly without being limited by normal tissue toxicity, until every cancer cell is eradicated.

Cytokinesis

Cytokinesis is the final stage of cell division during which separation of the two daughter cells created by the mitotic process occurs [33,34]. Myosin II is the principal motor for cytokinesis using the energy derived from the hydrolysis of ATP to move along actin filaments, causing contraction of the contractile ring and ultimately splitting the cell into two daughter cells. PKM2 [35], but not PKM1 [36] has been reported to play a critical regulatory role in cytokinesis by binding to and phosphorylating the spindle checkpoint Bub3
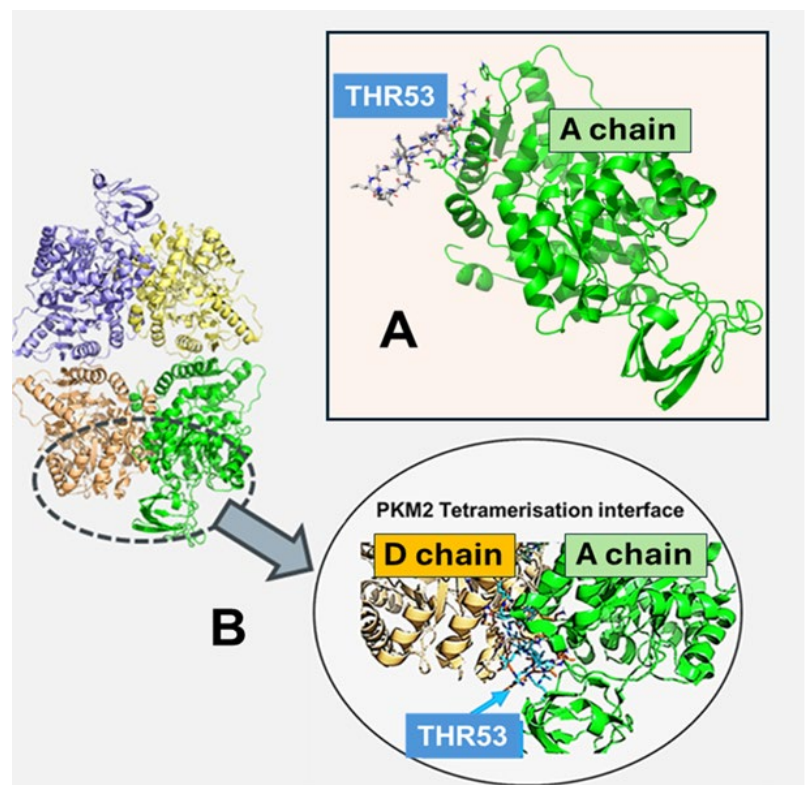
Figure 3. Simulated docking of PRGPRP warhead in a cyclic amphiphilic cassette (THR 53) with tetrameric PKM2. The THR 53 peptide {⌐FPPRGPRPVLKLALKLALK⌐Cyc} was built using Rosetta. Simulation with enhanced sampling was used to obtain stable solution structures; Docking simulations were performed with and without amino acid restraint. **A.** Binding to A chain with restraint. **B.** Binding to A chain overlayed with D chain.
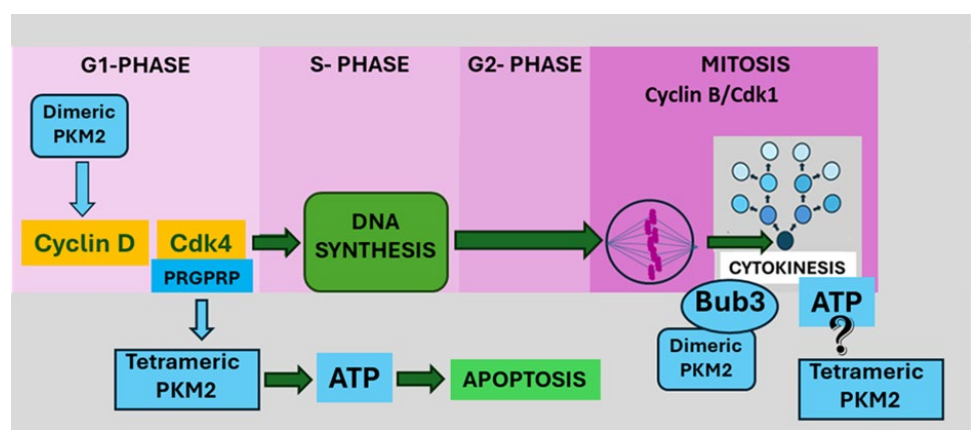

Figure 4. Putative dimeric and tetrameric PKM2 activity in relationship to the classical cell cycle. PKM2 is an active participant in its dimeric form in the signaling control of Start and in its tetrameric form in the energy supply of apoptosis in the G1 phase of the cell cycle. It has also recently been identified as a signaling molecule in the final step of mitosis [36] and here it is proposed to also, in its tetrameric form, provide the energy in the form of ATP required for cytokinesis.

at Y207 required for the Bub3-Bub1 complex to interact with Blinkin [37]. PKM2 also interacts with the light chain of myosin II (MCL2) [38,39].

The signaling function of PKM2 involved in the control of cytokinesis at the Bub3 site is likely to be carried out by dimeric PKM2, but as yet no mechanism has been provided to explain the source of the energy required for the cleavage required to separate the mitotic parent cell into two new daughter cells. It is proposed that in addition to its dimeric conformation as a regulatory signaling molecule, PKM2 at this site can also assume its tetrameric motor conformation. Tetrameric PKM2 would provide the ATP required to carry out the physical process of separation of the two daughter cells that completes cytokinesis. Inhibition of ATP production by tetrameric PKM2 by a PRGPRP bearing molecule such as THR53 at this critical final phase of cancer cell division would result in death. This would provide an explanation for the morphological appearance of THR-53 treated H460 Human Non-small cell lung cancer cells immediately after failed mitoses as seen on time lapse video photomicrography (**APPENDIX video: Right Hand Panel**).

The Limitations of Targeting Somatic Mutations

Present targets used for the design of anticancer treatments are the result of somatic mutations which cause the relative overexpression or activation by modifying structural alterations of normal cellular molecules, such as signal transduction factors, cell surface receptors and those neoantigens recognized by the immune system. By contrast PKM2 expression rather than PKM1 expression provides a unique phenotype with an absolute rather than a relative difference between cancer and normal in which reliable targets for uniquely selective anticancer agents can be sought.

Notably, because expression of the PKM2 isoform is not the result of mutation it should provide a stable target uninfluenced by the heterogeneity and genetic instability that is characteristic of mutation induced targets. Somatic mutations are stochastic events, and as such, their distribution and penetrance will be random across any field of cancer cells.

Observations of tissue sections of typical cancers stained immunohistochemically for expression of HER2 for example, show a random pattern of fluorescence intensity from cell to cell [40]. Some cells show high expression of the HER2 receptor, a few show little or no expression and the majority lie in between.

Such uneven penetrance means that a therapeutic drug targeted at a specific oncogene is likely to kill cells with the highest penetrance leaving those with lower or no oncogene expression to survive and repopulate to give rise to a resistant cancer (**Figure 5**). A similar argument applies to the neoantigens induced by mutations, against which the body mounts an immune response. It is not surprising, therefore, that widespread therapeutic induced acquired resistance is sufficiently common in oncology to merit the title of a hallmark of cancer.

Spontaneous Cancer Cell Death

Unrestrained cell division is widely acknowledged to be the key hallmark of cancer leading to the general belief that cancer cells are immortal. The fundamental assay of
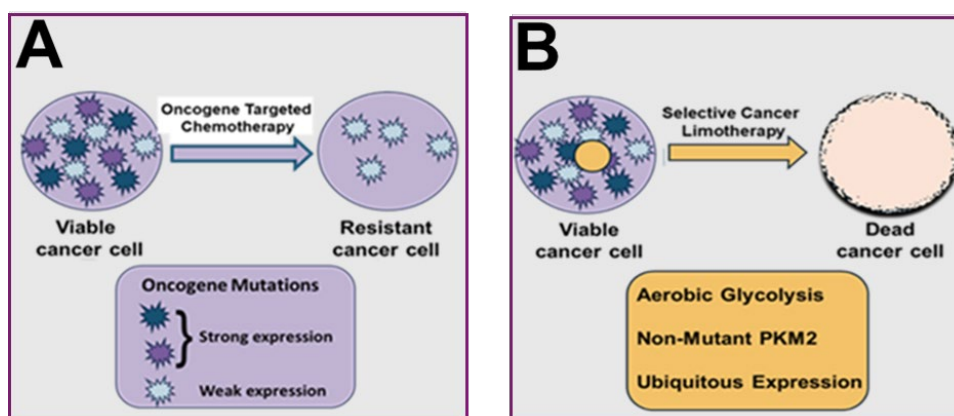

Figure 5. Comparison of oncogenic and aerobic glycolytic therapeutic targets. A. Mutation induced oncogene targets exhibit variable phenotypic expression. Targets with the lowest phenotypic expression are less likely to result in cell death leading to the development of acquired drug resistance. **B.** PKM2 is a non-mutant aerobic glycolysis target resulting from isotypic expression of the normal wild-type pyruvate kinase gene and is a homogeneous target which will consistently respond to selective SCCL treatment even though the mutated targets within the cancer cells respond variably.

unrestrained cancer cell division is a clonogenic assay which measures the ability of a cancer cell to go through repeated divisions creating daughter cells each of which are capable of further unrestrained divisions [41]. Despite the development of optimal tissue culture conditions for human cancer cell lines since the middle of the last century and the improved ease with which the human cancer cell lines themselves have adapted to *in vitro* growth, there still remains a wide variation in the percentage of single human cancer cells which give rise to colonies in clonogenic assays. This variability is reflected in the "plating efficiency", a value obtained for an individual cell line reflecting the percentage likelihood of clone formation from a single cell.

Serial photo microscopic studies of different human cancer stem cell lines growing under optimal growth conditions in tissue culture clonogenic assays reveal considerable differences in the percentage of cells able to successfully accomplishes the requisite number of consecutive cell divisions to form an intact colony of over fifty daughter cells. It is therefore necessary to normalize the results of therapeutic clonogenic assays to the relative plating efficiency of each cell line when comparing different treatments. The morphological appearances of the dying colonies in hematoxylin and eosin light microscopy images of untreated clonogenic assays (**Figures 6A** and **6C**) are strikingly similar in appearance to phase contrast images of human cancer cell lines in which exposure to the ONKONEK analogue, THR53, has induced a drop in intracellular ATP causing necrotic death (**Figures 6B** and **6D**) [32]. The plating efficiency of control untreated human cancer cells in clonogenic assays across a range of different untreated cell lines has been measured as being between 25% and 71.7% with a mean of 50.1%. It appears, therefore, that around half of human cancer cells undergo spontaneous cancer cell death by necrosis because they run out of energy when attempting to form colonies by repeated divisions of cancer stem cells. This spontaneous attrition of energy in a cancer undergoes further depletion following treatment with an anti-metabolic agent such as THR-53 or ONKONEK, that targets aerobic glycolysis causing even further ATP depletion providing an explanation of the similar morphological appearances between spontaneously dying untreated cells and cells exposed to THR-53 seen in H460 Non-small cell lung cancer (**Figures 6A** and **6B**) and SW320 colorectal cancer (**Figures 6C** and **6D**). Cancer cells should thus not be viewed as super-cells which outperform normal cells so much as damaged cells prone to spontaneous cell death by necrosis which makes them vulnerable to anti-metabolic agent attack by agents targeting aerobic glycolysis. The cancer cells die because, driven by mitotic addiction, they cannot avoid entering cytokinesis although they have insufficient ATP to complete it successfully.

Mitotic Addiction

The term oncogene addiction describes the process in which cancers with genetic, epigenetic, or chromosomal irregularities become dependent on one or several genes for maintenance and survival. As a result, cancer cells rely on continuous signaling from these oncogenes which make them desirable targets for new chemotherapeutic agents [42]. Cancer cell autonomous proliferation, however, is not only dependent on addiction to a relatively small group of dominant oncogenes, but also relies on many other phenotypic factors including inactivation of suppressor genes and the non-mutation-induced isotypic expression of normal PKM2 responsible for aerobic glycolysis. The principal difference between cancer and normal cells, therefore is that, further to addiction to oncogenes, cancer cells are addicted to mitosis itself whose critical final step of cytokinesis (**Figure 4**) is suggested here
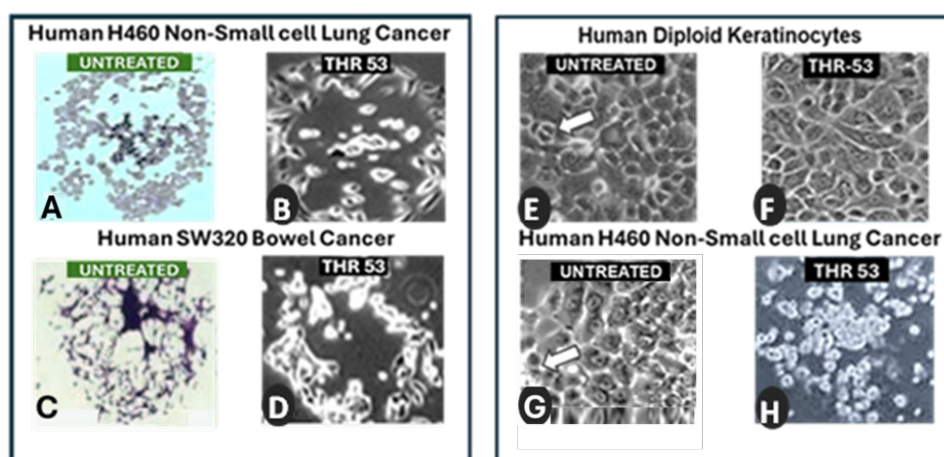

Figure 6. Photomicrograph appearances of spontaneous and selective antimetabolic induced cancer cell death.

to require ATP generated by tetrameric PKM2 located at the dimeric PKM2 binding site to Bub3 and myosin II. In this way an anti-metabolic therapeutic attack on aerobic glycolysis selectively kills cancer cells by inhibiting mitotic cell division.

Mitoses are a prominent feature of typical human tumors growing in tissue culture, often seen as doublets under phase microscopy (**Figure 6G arrow**), which having replicated their genetic code detach from the substratum whilst undergoing the physical process of fission to yield two new daughter cells each containing a new copy of the original genome. Repopulating stem cells in normal tissues seen in short term culture of normal human diploid keratinocytes show mitoses of similar, though more regular, appearance (**Figure 6E arrow**). ATP cannot be stored easily within cells [43] and human keratinocyte stem cells exposed to a PRGPRP congener undergo cell cycle arrest showing a flattened appearance with no evidence of mitoses (**Figure 6F**). Normal stem cells do not therefore proceed into mitosis and so do not become vulnerable to killing by PKM2 inhibitors during cytokinesis. Human *in vitro* cancer cell lines such as H460 Non-small cell lung cancer, exposed to homologues of the wild-type Cdk4 C'-terminal hexapeptide PRGPRP are unable because of their mutated signaling systems to stop dividing and proceed into mitosis terminating in a cytokinesis lacking sufficient ATP for successful completion.

Thus, although normal stem cells and cancer cells both exhibit aerobic glycolysis, anti-metabolic agents containing warheads such as PRGPRP, targeted at down-regulating PKM2 are able to selectively kill cancer cells by depriving them of the energy needed to complete a successful mitosis whilst only causing a pause in the cell cycle in normal stem cells. Attacking aerobic glycolysis thus appears to be a subtle way of attacking the unique tendency of the cancer as opposed to the normal cell, to undergo unrestrained mitotic division.

Conclusion

Cancer cells can be best understood as hybrids, composed of a somatic mutation-induced immortal phenotype and a normal aerobic glycolysis phenotype. Chemotherapy has been designed in the past to attack cell division by causing irreparable damage to the molecules involved in DNA synthesis and, where it is still frequently used, can only be given for a limited number of courses because of accumulating damage in normal cells. Around 568 different potential targets have now been described as resulting from somatic mutations affecting the on and off signals controlling cell division and creating the immortal cancer phenotype [44]. Rapid advances in DNA sequencing in cancer have led to the potential approach of personalized therapy by selectively targeting specific mutations responsible for increased cell division in individual patients with less damage to normal cells. These mutated targets suffer, however, from uneven heterogenous phenotypic expression and target disappearance due to

genetic instability which are likely to lead to drug resistance. Similar constraints apply to the neoantigens on the surface of cancer cells which are targeted by the body's own immune system. Further therapeutic advances in cancer would benefit from druggable non-mutant stable targets. It has been suggested that the PKM2 isoform of the pyruvate kinase gene responsible for aerobic glycolysis, which is found ubiquitously throughout malignant cells, could provide such a target, making it the true "Achilles Heel" of cancer. Spontaneous cell death in cancer cells but not normal cells make them already more vulnerable than normal cells to energy depletion which would provide targeting aerobic glycolysis in general with a selective advantage. More specifically targeting ATP production by PKM2 deprives cancer cells driven into cytokinesis by mitotic addiction of the required energy to complete division into two daughter cells.

The whole person dietary approach of limotherapy, has not infrequently been proposed as a way of treating cancer [45] but it applies to the whole soma of an intact individual, both cancer and normal and does not comply with the desired dictum "The tumor should shrink faster than the patient". Here a new therapy is described that applies limotherapy exclusively to the cancer cells but not to adjacent or distant normal cells within the body. Non-nucleate mature erythrocytes which like cancer cells depend on aerobic glycolysis should not be affected by this treatment because they use the PKR enzyme which does not contain the same SDPTEA target as PKM2.

The discovery that the unique hexapeptide sequence PRGPRP in an external loop of the C'-terminus of Cdk4 modulates the production of ATP by PKM2 has led to the development of ONKONEK [18,32], a first in class example of the new therapeutic paradigm of selective cancer cell limotherapy, a global cancer therapeutic approach which does not damage normal cells but depletes ATP in cancer cells. The putative site of action of PRGPRP congeners at the final cytokinetic step of mitosis provides a role for aerobic glycolysis in the context of the cell cycle and an explanation for why antimetabolic therapy can lead to the death of dividing cancer cells. Because the new therapeutic approach can potentially kill individual cancer cells within the body by intermittent starvation rather than by generalized irreparable molecular damage it has the potential to be administered repeatedly for as long as is necessary until the very last cancer cell is eradicated.

Ethics

No ethical approval required.

Clinical Trials

None.

Data Availability

All data is in earlier literature and in patent applications except new unpublished structural chemistry data in Figure 3.

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