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The Role of DNA synthesis imaging in cancer in the era of targeted therapeutics

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Abstract

Non-specific targets such as DNA and microtubules have been the mainstay of cancer therapeutics and the most effective clinical agents until a decade ago. Advances in genetics, molecular and cellular biology over the past decade led to the development of a new generation of agents that are far more specific and effective. In contrast to progress seen with therapeutic agents, general monitoring targets such as proliferation imaging are just gaining momentum and targeted imaging is still in its infancy. In these paradoxical times, this review assesses the role of proliferation imaging in monitoring the efficacy of targeted therapeutics.

Keywords

Proliferation; DNA synthesis; Thymidine kinase; THymidylate synthase; Thmidine phosphorylase; Thymidine; FMAU; FBAU; FAU

INTRODUCTION

Over the past decade, the understanding of cellular and molecular biology in association with genomic and proteomic approaches have led to the identification of crucial pathways in cell proliferation and metastasis. This has led to the development of targeted therapeutic agents with greater efficacy. However, the ability to monitor the efficacy of targeted therapies in the development stage still remains a challenge. One important way to monitor targeted therapies is to observe the target expression levels. This may not be feasible in all situations and is more striking in a clinical setting where combination therapies are frequently used. In such an environment, it is not only the function of the main target that is affected but also every step in which the target protein is implicated, with often unpredictable consequences regarding the final biological effects. In a realistic situation, it may not always be feasible to assess the efficacy of a combination treatment based on a single targeted imaging agent. This could be circumvented by understanding the effector pathways of these combination treatments or through the use of generalized monitoring methods such as metabolic or proliferation imaging.

This review will focus on imaging agents for cellular proliferation and their role in monitoring the efficacy of various targeted therapeutic agents in pre-clinical as well as clinical studies.

1. IMAGING PROLIFERATION

Cellular growth can be measured in many ways, for example using labeled amino acids [1], lipid precursors [2] and nucleosides [3, 4]. Both amino acids and lipid precursors tend to have more than one metabolic pathway. Nucleosides, particularly thymidine and its analogs, are unique because of their involvement in the DNA salvage pathway. This review will specifically address the rationale for imaging the DNA salvage pathway, the enzymes involved, current imaging probes and their role in predicting or monitoring the therapeutic efficacy of targeted therapeutic agents.

1.A. HISTORIC PERSPECTIVE AND RATIONALE

Once the structure of DNA was elucidated in 1952 it became clear that DNA synthesis and the use of nucleosides in this process was linked to cell proliferation. Radioisotopes were initially used to help study the pathways involved in production of the nucleotides, nucleosides, and RNA and DNA. Once the pathways had been studied it became clear that these radiolabeled bases could be used to help monitor and understand cellular proliferation. Thymidine labeled with either ^3H or ^{14}C became a standard measure of cell growth since thymidine, of all the nucleic acid bases, was the only one that was not incorporated into RNA. Hence, retention of this labeled base reflected the level of DNA synthesis and cell replication, while cytosine, adenosine and guanosine could also end up in RNA after replacement of the deoxyribose. The advent of PET imaging led investigators to consider the use of ^{11}C -thymidine as an imaging agent for cellular proliferation. The synthesis of this compound by Wolf and colleagues started investigators on the path of imaging DNA synthesis [5].

To use labeled thymidine for imaging requires a detailed knowledge of the steps in DNA incorporation and an understanding of the clearance and metabolism when thymidine is used as a tracer. Thymidine utilization in tissues had been extensively studied over the years in cell culture, animals, and even in a limited number of patients [6–8]. The application of this knowledge to interpreting images, however, must take into account the differences in the approaches to measuring thymidine retention. Many of the studies in cells and animals have measured the retention of the radiolabel, usually using long-lived ^3H or ^{14}C -thymidine, after the extraction of the tissue. These extraction processes usually removed small molecules, including thymidine itself and its metabolic breakdown products, from macromolecules such as DNA. This was often done by acid precipitation of the DNA from mixtures of disrupted cellular contents. Studies were also performed using fixed tissues, where fixed cells retained labeled DNA. Washing of preserved tissues generally removes small molecules. Hence, when measuring the number of cells that have incorporated labeled thymidine into their DNA, as is done with autoradiography of fixed tissue slices, the contribution of unincorporated thymidine is removed.

Unfortunately, imaging with PET cannot distinguish the molecular form of the radiotracer detected. All of the radioactivity present in a given volume element is measured with PET, irrespective of the molecular entity which is represented. One must choose labeled compounds that are trapped by pathways of interest, so that the presence of a concentration above background reflects specific retention, while an untrapped tracer or its metabolites are clearing from the tissue and may contribute to a nonspecific background. The following section will describe the enzymes involved in trapping the proliferation tracers and the

advances in metabolically stable proliferation imaging agent development and their utilization.

2. TARGET ENZYMES

2.A. Cytosolic thymidine Kinase (TK1)

TK1 (EC 2.7.1.21) is a key enzyme in the one-step salvage pathway of pyrimidine biosynthesis. TK1 catalyzes the transfer the γ -phosphate group of ATP to the 5'-hydroxyl group of Thd and dUrd resulting in Thd-phosphate in a Mg^{2+} -dependent fashion. Expression of TK1 is strictly cell cycle regulated. Its activity increases markedly after the G1-S transition and then declines rapidly in G2. The active enzyme found in S-phase coordinates with DNA replication [9–12]. Upregulation of human TK1 activity was observed in several cancers and proposed as a prognostic marker [13–16]. TK1 expression is regulated by E2F, a cell cycle regulated transcription factor [17]. Due to a high proliferation rate, activity within cancer cells often expresses high levels of TK1, which can be used for the detection of tumors and metastases by means of positron labeled tracers [18].

2.B. Thymidylate Synthase (TS)

Thymidylate synthase (TS, (EC 2.1.1.45),) is a key enzyme acting in the de novo synthesis of pyrimidines. TS catalyzes the reductive methylation of dUMP by N5,N10-methylene tetrahydrofolate. The product of TS catalysis is 2'-deoxythymidine-5'-O-monophosphate (dTMP). TS is required for the de novo synthesis of dTMP. dUMP, derived from either the deamination of dCMP (catalyzed by dCMP deaminase), or from the hydrolysis of dUTP (catalyzed by dUTPase) under goes reductive methylation by N5,N10-methylene tetrahydrofolate. The product of TS catalysis, dTMP, is readily phosphorylated to dTTP, one of the four principal building blocks required for DNA synthesis [19]. Hence the inhibition of TS effectively limits DNA replication and cell division, a rationale behind the development of chemotherapy drugs, such as 5-fluorouracil (5FU) and methotrexate. Over-expression of TS has been linked to resistance to these agents both in vitro and in vivo [20, 21]. High levels of TS expression have also been correlated with poor prognosis in breast cancer [22], gastric cancer and colorectal cancers [23]. Imaging TS resistance or the development of imaging or therapeutic agents that will utilize the TS over expression levels is of interest.

2.C. Thymidine Phosphorylase (TP)

TP(EC 2.4.2.4) drives the salvage pathway and therefore plays a critical role in maintaining the balance of the nucleotide pool and nucleic acid homeostasis by ensuring the correct supply of deoxyribonucleoside triphosphates (dNTPs) for DNA replication and repair [24, 25]. The main function of TP is catabolic; and catalyzes the phosphorolysis of the nucleosidic linkage of pyrimidine 2'-deoxynucleosides with the formation of the pyrimidine and 2-deoxy-a-D-ribofuranose-1-phosphate [26]. The phosphorolysis reaction is reversible hence the catabolic function of TP is inhibited by thymine and enhanced by thymidine. To prevent the TP associated catabolism, nucleoside analogs with greater stability are developed by substituting the 2' or 3' hydroxyl group of the sugar with a fluoride. TP is identical to platelet-derived endothelial cell growth factor [27, 28]. TP activity has been demonstrated to be higher in esophageal, stomach, colorectal, pancreatic and lung carcinomas compared to normal tissue. Expression levels of TP have been used both for the activation of prodrugs and as a prognostic marker [29]. There have been considerable interests in TP imaging yet research efforts have resulted only in a limited development of imaging agents [30–32].

3. IMAGING AGENTS

3.A. Thymidine kinase based imaging agents

Most of the proliferation imaging agents developed to date primarily target cytosolic thymidine kinase (TK1). Preclinical and clinical studies [6–8] of thymidine labeled with ^{11}C (20.2 min) at methyl or ring-2 positions showed rapid catabolism generating considerable amounts of recirculating radioactive metabolites. The radioactive metabolites contribution to images demand extensive kinetics modeling [33–36]; making ^{11}C -thymidine impractical for routine clinical use. Attempts to develop bromodeoxyuridine (BUdR) [37–39], as a PET in vitro tracer for proliferation, was less successful due to its metabolism and dehalogenation, resulting in recirculating ^{76}Br -Bromide. Similar dehalogenation problems were also observed in the development of iododeoxyuridine (IUdR) [40–42] as a proliferation imaging agent.

These limitations led to the development of more stable nucleoside analogs that could be labeled with longer half-life radionuclides. Nucleosides with a substitution of fluoride at the 2' or 3' position (Figure 1) of the pentose sugar are more resistant to degradation by thymidine phosphorylase in addition to offering a quality labeling choice for clinical PET studies. One most promising agent with a 3' substitution is 3'-deoxy-3'-[^{18}F] fluorothymidine (^{18}F -FLT). It is resistant to degradation and trapped intracellularly after phosphorylation [18]. FLT is phosphorylated by cytosolic thymidine kinase (TK1) (Figure 2). FLT uptake correlates well with tumor cell proliferation and S-phase fraction [43–45].

3.B. FLT

FLT-PET has been used to monitor proliferation changes in different cancers both at the preclinical and clinical levels in various therapeutic regimens. The following examples will illustrate FLT-PET use in monitoring conventional or targeted chemotherapy as well as radiotherapy.

Conventional chemotherapies primarily target general pathways such as DNA replication, microtubulin polymerization or tubulin heterodimers etc., and therefore result in overall reduced cell proliferation or apoptosis. Leyton et al., demonstrated changes in proliferation after the common chemotherapeutic agent cisplatin treatment could be detected by FLT in a radiation-induced fibrosarcoma model [46]. The authors demonstrate that changes in FLT uptake is due to G1-S and G2-M phase cell cycle arrest and changes in thymidine kinase 1 levels. In comparison to glucose metabolic changes, they determined that proliferation change detection is rapid and superior. Oyama et al., used FLT to image proliferation changes in an androgen-dependent prostate tumor model. The authors demonstrated that androgen ablation, caused by diethylstilbestrol treatment, induced proliferation changes that could be detected in a CWR22 prostate tumor model [47].

Radiation induced proliferation changes could also be monitored by FLT imaging. IR activates the G1, G2 and S phase check points and inactivation of E2F transcription factor [48]. Therefore TK1 levels regulated by E2F are also reduced and could be monitored by FLT [17, 49]. Supporting this concept, Sugiyama et al. have demonstrated that uptake of FLT and changes in FLT uptake after radiotherapy and photodynamic therapy were more pronounced than FDG and correlated well with proliferating cell nuclear antigen (PCNA) labeling index [50]. Radiation induced proliferation changes were detectable as early as 3 hrs post treatment unlike FDG. In another study, Apisarnthanarax et al. demonstrated the use of FLT in detecting changes in proliferation after chemoradiotherapy using docetaxel followed by irradiation in an experimental SEG-1 human esophageal carcinoma model [51]. Similarly, Yang et al. have also employed FLT to assess early response to radiation in a xenograft model and compared it to changes in FDG uptake. This study found a marked

FLT response to single doses of radiation at early time points (1–2 days post-therapy) which were larger than those measured with FDG-PET [52].

FLT has also been used to monitor the proliferation changes caused by several targeted therapies ranging from epigenetic modulators to signaling cascade inhibitors. Aberrant histone deacetylase (HDAC) activity leads to transcriptional repression of tumor suppressor genes, a general contributor to tumor formation. Thus, HDAC inhibition has generated considerable interest and HDAC inhibitors are in Phase I clinical trials [53]. One such inhibitor is LAQ824 [54]. In a recent study, Kenny et al., demonstrated that LAQ824 treatment results in reduced transcriptional and translational activity of TK1, which was used to measure the therapeutic effectiveness in an animal model using [F-18]FLT [55].

EGFR and its family of Erb-B cell surface tyrosine kinase receptors are often overexpressed and associated with aggressive phenotype. Due to their expression level and biological roles in cell proliferation through MAPK, JNK and Akt pathways, Erb-B tyrosine kinases have been a therapeutic target [56]. A second generation of pan Erb-B kinase inhibitor CI-1033 is in phase I/II clinical trial [57, 58]. Dorow et al., have used a multi-tracer imaging approach to image the pharmacodynamic effects of Pan Erb-B kinase inhibition by CI-1033. The authors demonstrate that CI-1033 treatment resulted in a 35% reduction in Ki-67 staining as well as TK1 activity after 6 days and the changes in these proliferation rates could be non-invasively measured by FLT-PET [59].

Unlike Erb-B family, aurora kinases, a family of serine/threonine kinases, are directly involved in mitotic process of cell division and therefore control proliferation. Aurora kinases are of therapeutic interest due to overexpression and the association with genetic instability in tumors [60]. In a recent study, Chan et al., illustrated that Aurora kinase inhibitor CCT129202 induces a p21 and results in low TK1 thymidine kinase expression levels through Rb1 pathway [60]. These inhibitor modulated TK1 expression levels were utilized to noninvasively monitor the aurora kinase targeted therapy by FLT-PET [61].

3.B.1 FLT Studies in Patients—For nearly a decade FLT-PET imaging has been used in detection and therapeutic monitoring of several cancers. The initial imaging studies done in patients demonstrated similarities and some important differences in the metabolism and retention of FLT in humans compared to animals [18]. Tumor uptake of the tracer was nicely demonstrated in human tumors as well as in the bone marrow. While marrow uptake is expected, since it is a rapidly proliferation tissue, it does limit the ability to detect metastatic lesions to the bones. On the other hand, FLT also demonstrated increased uptake in the liver, while this had not been seen in studies of other mammals. This reflects the glucuronidation of FLT, which is not seen in dogs or rodents [62, 63]. This limits the ability to detect tumor metastases in the liver, but some tumors may be seen above the normal hepatic background. The glucuronidation of FLT must also be taken into account when kinetic modeling is done, since by 60 minutes post-injection about 25% of the labeled blood activity is present as the glucuronide [64, 65]. Little cardiac uptake of FLT is detected unlike that seen with ^{11}C -thymidine in humans. Cardiac uptake of thymidine is due to mitochondrial thymidine kinase 2, but FLT is not a good substrate for TK2, limiting retention in this organ [66]. Clearance of FLT by the kidneys and into the bladder was also noted in the initial studies.

Because of the low background activity of FLT in the thorax a number of studies have explored the use of FLT in lung cancer and some have included breast cancer [67–69]. Studies in the abdomen have been fewer, in part due to the limitations of physiologic uptake by the liver, kidneys, and bladder. FLT uptake by lung tumors correlated well with tumor proliferation as measured by histochemical assessment of tumor Ki-67 levels with $r=0.92$,

$p < 0.0001$ and $r = 0.84$, $p = 0.0011$ in studies by Buck and Vesselle, respectively [70, 71]. The intensity of FLT retention is generally lower than that seen with FDG, hence FLT does not appear to have any great advantage in the detection or staging of lung cancer. For example in one study the mean FDG and FLT SUV were 4.1 and 1.8 respectively [70]. A recent study demonstrated that the sensitivity of FLT and FDG was 72% and 89%, respectively [72]. The improved correlation with proliferation, however, suggests that FLT retention may be a better measure of tumor response to treatment (Figure 2). In a recent study, patients with lung or head and neck tumors were imaged with FLT before and about 2 weeks after the start of radiation treatment [73]. FLT SUV declined about 20–50% in the tumors and there was complete bone marrow ablation. On the other hand, treatment with anti-metabolites such as gemcitabine may lead to an increase in FLT retention within a day of treatment [74]. This may be the result of the cells changing pattern of TK1 expression.

FLT has also been shown to demonstrate increased uptake in head and neck tumors, but while it had excellent sensitivity in a pilot study (10/10 patients), the specificity was low (16.7%) [75]. This was the result of FLT retention in reactive lymph nodes, which has high Ki-67 staining. On the other hand, in esophageal cancer FLT had better specificity than FDG, and only missed nodal metastases in one patient (10 patients studied) [76]. In this study, neither FLT nor FDG retention correlated with the level of tumor proliferation as measured by Ki-67 staining. Because of the low background of FLT in the thorax, FLT is also able to readily detect breast tumors [77]. While the SUVmean was a little lower with FLT (3.2) compared with FDG (4.7), the tumor-to-background ratio was actually higher with FLT. FLT has also been shown to be useful in monitoring treatment response to chemotherapy in pilot studies [78, 79]. The decline in FLT retention after the first cycle of chemotherapy was found to correlate with change in tumor size a few months later ($r = 0.74$, $p = 0.01$) [79]. In another study the decline in FLT one week after the start of therapy was found to predict response two months after the start of therapy [78].

FLT has demonstrated increase retention in non-Hodgkin's lymphoma, it can be used to separate aggressive from indolent tumors, and FLT retention correlates with Ki-67 index [80, 81]. A recent study has evaluated the use of FLT in the treatment of patients with high-grade non-Hodgkin's lymphoma. In patients treated with rituximab and cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP) chemotherapy there was a decline of 77% in FLT SUV by seven days after treatment and slight further decline of 85% from baseline by day forty [82]. Rituximab alone did not cause a significant change in FLT retention in two days, but CHOP decreased FLT SUV by 32% in the same interval. The early declines in FLT retention were predictive of patients who would eventually attain a complete response, compared to those who were only partial responders. The evaluation of residual masses after treatment of Hodgkin's and non-Hodgkin's lymphoma demonstrates that FDG and FLT are both useful, but that FLT may provide more information about survival [83].

While FLT may prove to be very useful in non-Hodgkins's lymphoma, a recent study in rectal cancer did not appear as promising [84]. Patients with rectal cancer were imaged two weeks after the start of neo-adjuvant chemoradiotherapy and then again three to four weeks after completing the treatment, but before surgery. On average FLT retention declined by 28.6% and 54.7% at these time points. In this pilot study of ten patients, the degree of decline did not correlate with histopathologic response in that those with $<10\%$ viable tumor had a mean decline of 55.6%, while those with $>10\%$ viable tumor had a decline of 53.7% at the end of treatment. There was also no difference at the early time point.

FLT imaging of brain tumors had been an active area of research. Thymidine and its analogs do not normally cross the blood-brain barrier, resulting a very low background. Imaging with FLT in the brain is limited to areas of increased permeability, as reflected by

enhancement on contrast MRI [85]. Hence tumors can readily be seen because of their high tumor-to-normal tissue ratios (about 3.85), even though their uptake may be relatively low (SUVmax 1.33) [86]. As a result of the high background seen with FDG, FLT provided improved contrast with high grade but not low grade brain tumors [87]. FLT may be useful in the detection of recurrent tumors, but one must be careful that benign lesions with breakdown of the blood-brain barrier are not misinterpreted [88, 89]. FLT was found to be useful in discerning malignant brain lesions that respond to treatment [90]. A study of nine patients using a three compartment model describes the fraction of transported FLT that is phosphorylated and findings correlated with pathology. It was also noted that the SUV and FLT influx rate were strongly correlated, so the simpler approach to quantitation may be sufficient.

3.C. FMAU and FBAU

Although FLT is very promising as a proliferation imaging agent, it is not readily incorporated into DNA and therefore does not reflect the total DNA synthesis. Examples of a 2' substituted analog that could be incorporated into DNA ^{11}C [91, 92] and ^{18}F labeled FMAU [93, 94]. FMAU, similar to thymidine, is phosphorylated by thymidine kinases and incorporated into DNA by DNA polymerases. A schematic representation of the activation of various nucleoside analogs is described in Figure 1. In vitro studies have shown that the amount of FMAU incorporation into DNA is proportional to DNA synthesis [95]. *In vivo* studies in normal dogs using ^{18}F -FMAU showed substantial quantities of radioactivity in the acid precipitable large molecular fraction suggesting FMAU incorporation into DNA [93]. In addition, ^{18}F -FMAU studies in humans indicate a clear visualization and uptake of activity in tumors of the brain, thorax, prostate and bone indicating that it might be useful as a direct marker for monitoring DNA synthesis [94].

Recently, our lab and others have synthesized and studied ^{76}Br or ^{18}F labeled 1-(2'-deoxy-2'-fluoro-1- β -D-arabinofuranosyl)-5-bromouracil (FBAU) as a proliferation marker [96–101] in mice, rats and dogs. The rationale for studying FBAU is that, similar to FMAU and other thymidine analogs, it will be phosphorylated by cytosolic thymidine kinases and subsequently incorporated into newly synthesized DNA. Such cellular trapping of the tracer will be a good indicator of DNA synthesis and simultaneously offers a labeling choice of a long lived isotope (^{76}Br ; 16.7 hrs) for protracted studies.

Both FMAU and FBAU are relatively poor substrates to TK1 in comparison to FLT or thymidine which may reflect in *in vivo* studies and low tumor to background ratios [101]. In dogs and humans thymidine and FLT produce images with high marrow uptake, reflecting the proliferation in this tissue. While FMAU uptake is seen within the marrow of dogs, we have not seen uptake within the human marrow [93, 94]. Our recent studies using human cell lines in culture demonstrate that FMAU retention increases as cells are stressed, while FLT retention declines in the same setting [102]. This reflects the phosphorylation of FMAU by mitochondrial TK2. Further work in this area is required, but FMAU and FBAU may provide early measures of treatment response because of increases in cell stress, rather than declines in DNA synthesis.

4. Thymidylate synthase imaging

Unlike TK1, direct imaging of TS expression has been limited in spite of its importance in clinical outcome. Initial studies were done to assess the TS inhibition using ^{11}C -thymidine imaging. ^{11}C -Thymidine imaging was used based on the observation that TS inhibitors reduce the endogenous TTP pools and cells compensate by incorporating extracellular thymidine pool for TTP synthesis [103–105]. Using ^{11}C -thymidine PET imaging, the authors demonstrate the feasibility of monitoring TS inhibition, an increase in tracer uptake,

caused by AG337 noninvasively in gastrointestinal cancer patients. While this study illustrates a new strategy for TS imaging, the use of ^{11}C -thymidine is complicated as described in previous sections. The development of new stable DNA synthesis imaging agents may offer an opportunity to revisit the old problem. Using an innovative approach, Perumal et al., have utilized the redistribution of ENT1 nucleoside transporter to the plasma membrane to image the TS levels with FLT-PET imaging. The authors demonstrate that the changes in 5FU indicated TS levels could be non-invasively monitored and the mechanism of uptake and retention is different from that of ^{11}C -thymidine [106].

Direct imaging of TS expression levels is still a challenge. TS levels are a predictive marker of 5FU, a thymidylate synthase inhibitor, treatment outcome in pancreatic and respectable colorectal cancers [21, 107–109]. [^{18}F]5FU has been synthesized and tracer kinetics have been extensively investigated in liver metastatic lesions of colorectal carcinoma [32, 110]. However, correlation of 5FU activity retention with TS levels has not been performed and seems to be an interesting avenue to pursue considering the availability of the tracer.

Another agent that was proposed to non-invasively image TS levels is (1-(2'-deoxy-2'-fluoro- β -D-arabinofuranosyl)-uracil (FAU). FAU is being studied as a potential therapeutic and PET imaging agent. FAU was designed to take advantage of high TS profiles observed in breast, colorectal, head and neck cancers that become resistant 5FU treatment. Once FAU is taken up into cells, it is phosphorylated to FAU monophosphate (FAUMP) by thymidine kinase and then methylated by thymidylate synthase to FMAUP. After further phosphorylation, FMAU-TP is utilized in DNA synthesis [111]. So instead of inhibiting TS, a general drug targeting mechanism, FAU is activated by the high TS levels into a cytotoxic drug.

Studies have demonstrated that [^{14}C]FAU is incorporated into DNA as FMAU, and this appears to be its mode of cytotoxicity. After incubation of cell lines with FAU or FMAU, the percentage of thymidine replaced correlated with growth inhibition [95]. These *in vitro* studies were further supported by *in vivo* studies in mice showing the incorporation of FAU into DNA as FMAU [112]. This makes FAU a particularly attractive drug as well as an imaging agent, since tumors that are resistant to 5-fluorouracil (5FU) and other TS inhibitors are expected to be more sensitive to FAU toxicity. Preliminary studies with [^{18}F]FAU as an imaging agent indicated low concentrations of activity in proliferating tissues in dogs or humans, although modest increased uptake was seen in some tumors [113]. *In vitro* TK assays showed that FAU is a very poor substrate for mammalian cytosolic thymidine kinase (TK1) relative to thymidine or FMAU [114]. This preliminary data suggests that the efficacy of FAU is limited by low activation at the first phosphorylation step. While there is no guarantee that TS activation would not become another hurdle, a recent report [115] showing that TS activation is the most determinant step for incorporation of FAU into DNA has prompted us to develop FAU prodrugs to circumvent the poor phosphorylation of FAU by mammalian thymidine kinases.

5. Thymidine phosphorylase imaging

Thus far there have been very few, rather unsuccessful attempts in imaging the phosphorylase activity of thymidine phosphorylase. Recently, Grierson et al., developed 5'-deoxy-5'-[F-18]fluorothymidine ([F-18]DFT) as a tracer for intracellular TP expression. The tracer uptake did not show a correlative uptake with TP expression levels in A459 and U937 cell lines, reason being that initial metabolite generated undergoes further metabolism into a diffusible secondary metabolite allowing loss of activity from cells [116]. In another report, Fei et al., reported the synthesis of [^{18}F]capecitabine, a prodrug of 5FU which utilizes TP for

activation. However, the kinetics, distribution, and biological use of [^{18}F]capecitabine are yet to be reported [117].

As discussed in the above sections, the use of single or combination chemotherapeutic agent therapeutic efficacy could be monitored either based on cell cycle changes, variations in TK1 activity or other target protein expression levels involved in DNA salvage pathway. A few examples of commonly used chemotherapeutic agents either alone or in combination therapy and possible changes in TK expression levels are described in Table 1. Clearly studies are needed to demonstrate these theoretic expectations.

6. FUTURE DIRECTIONS

6.A. Proliferation, Non-invasive imaging and “-omics”

The advent of new inventions and molecular biology techniques allow high-throughput gene and protein expression to be achieved rapidly; therefore enabling the discovery and classification of cellular profiles [118–120]. These developments are aiding us in the prognosis of various malignant states and particularly the predictive markers that will most likely benefit a given patient [121, 122]. The discovery, refinement, and use of these molecular diagnostic tools will ultimately transform the practice of oncology into personalized medicine. As these promising technologies are being developed, the refinement of more traditional diagnostics is progressing and better results could be achieved by combining these expression assays with the traditional imaging technologies. Out of curiosity, we looked at the proliferation and metabolic processes associated genes in all the cellular process described in the cancer genome anatomy project Go browser. The result is that, not surprisingly, a considerable fraction of genes are associated with proliferation or cell cycle except for the protein metabolism (Figure 3). The percentage of genes associated with these various cellular processes may change as we progress through the genetic landscape of tumors. A closer look at the 40 published cancer microarray data sets of distinct tumor types by Rhodes et al., [123] and other studies convincingly demonstrates the uniformity of proliferation signature in tumor specimens [124]. Most of the tumor proliferation signature genes are often associated with cell cycle regulated genes during cell division of human cancer cell lines [125]. A comparison of He-La cell line and breast-tumor associated genes show that 62% of the breast tumor proliferation genes are cell cycle regulated. These studies not only demonstrate our ability to profile tumors on a genome scale and identify cancer-specific molecular changes but may also be useful in identifying what markers, particularly non-invasive, are reliable for early detection, therapeutic monitoring or predictive response. Also, this profiling will help in addressing what changes an imaging expert should expect with a specific drug or drug combinations. Combination of these molecular approaches with non-invasive imaging markers will arm us with functional profiling of molecular signatures.

Some of the molecular signature patterns could be determined even during preclinical assessment of potential drug candidates. Hence, a close interaction between the imaging community, oncologists and industry will help to streamline the evaluation of imaging markers by the time a potential therapeutic candidate emerges into the clinic, providing an opportunity for a decisive plan for non-invasive monitoring of therapy.

It is also likely that proliferation and or metabolic changes, due to significant variations in target expression levels, will be the next most suitable markers if a direct imaging of the target pathway is not available or does not appear to be driving tumor growth. It appears that the era of targeted therapeutics will likely rely on a combination of imaging of direct cellular drug targets, metabolic biomarkers, such as those for DNA synthesis, and conventional anatomic imaging for therapeutic assessment.

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Figure 1.

Pathway, enzymes and probes for proliferation imaging. Target enzymes are denoted by *italics*. Imaging agents that could be used to probe a specific enzyme are shown in the corresponding quadrant. TP specific imaging agents are not yet available. Abbreviations: TK, thymidine kinase; TP, thymidine phosphorylase; TS, thymidylate synthase; dUMP, deoxyuridine monophosphate; TMP, thymidine monophosphate; TDP, thymidine diphosphate; TTP, thymidine triphosphate, FLT, 3'-deoxy-3'-fluorothymidine; FMAU, 1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-methyluracil; FAU, (1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-uracil; FBAU, 1-(2'-deoxy-2'-fluoro-1-β-D-arabinofuranosyl)-5-bromouracil.

Figure 2. Monitoring treatment response by FLT. A and B) Coronal and transaxial [^{18}F]FLT-PET images of a lung cancer patient before treatment. Arrows indicate the tumor regions. SUV mean: 3.61 C). Transaxial image of the same patient after 7 weeks of chemo and radiation treatment. SUV mean: 1.20.

Figure 3. Metabolic gene signature in cancers. Each section of the pie chart represents the percentage of genes associated with the respective process as categorized in biological process of cancer genome anatomy project GO browser (<http://cgap.nci.nih.gov/Genes/GOBrowser>).

TABLE 1

Anticipated changes in TK1 expression activity due to chemotherapeutic treatments

Chemotherapeutic class/agent	Target	Cell cycle events	Anticipated early changes in TK1 activity
Flavopiridol	CDKs	G1/S and G2/M transition arrest	↓
Taxanes	Microtubules	G2/M arrest	↓
Camptothecins-Low concentration	Topoisomerase I	G2 arrest	↓
Camptothecins-high concentration	Topoisomerase I	S-Phase arrest	↑
Etoposide	Topoisomerase II	S or G2/M arrest	Variable
5-FU or TS blockers	TS	S-phase accumulation	↑
Methotrexate	Dihydrofolate reductase	S phase	↑
Cisplatin	DNA crosslinking	G1/S and G2/M arrest	↓
Gemcitabine	DNA Synthesis	Early S Phase arrest	↑
Combination treatments			
Irinotecan → 5FU	Topoisomerase I and TS	S-Phase delay	↑
5FU → 6-hr delay → Irinotecan	TS and Topoisomerase I	Synchronizastion of cells in S-pase	↑
5FU → UCN-01	TS, PKC and CDK	Suppression of TS due to E2F inhibition	↓

Abbreviations: CDK, Cyclin Dependent Kinase; PKC, Protein Kinase C