

# **FRONTIERS IN CELLULAR ENERGETICS, DIET & CANCER PREVENTION**

**National Institutes of Health  
National Cancer Institute  
Division of Cancer Prevention**

**Executive Plaza North  
Rockville, Maryland  
March 12, 2008**

## **Meeting Summary**

### **INTRODUCTION**

Nobuyo Tsuboyama-Kasaoka, PhD, RD, Guest Researcher in the Nutritional Science Research Group (NSRG) in the Division of Cancer Prevention (DCP) at the National Cancer Institute (NCI), opened the workshop on *Frontiers in Cellular Energetics, Diet and Cancer Prevention*. Dr. Tsuboyama-Kasaoka and Sharon Ross, PhD, MPH, Program Director in the NSRG, DCP, NCI Co-Chaired this workshop. Dr. Tsuboyama-Kasaoka provided brief background information on the focus of the workshop. She indicated that epidemiological evidence suggests an association between obesity and increased cancer risk at several sites including colon, ovary and breast. However, the interrelationship between obesity and increased cancer risk does not exist for all sites and the underlying reasons for this discrepancy are the subject of active debate among scientists.

Dr. Tsuboyama-Kasaoka outlined the workshop's objectives which were basically to enhance knowledge and understanding of the role of cellular energetics in cancer-related processes, as well as provide an overview of the evidence linking energy sensitive pathways, diet, obesity and cancer. Speakers were asked to address the role(s) of mTOR, IGF-1, AMP kinase and factors involved in their dysregulation during cancer as a function of dietary energy intake, exercise/physical activity and energy sensing pathways and the use of appropriate animal models.

Ms. Tsuboyama-Kasaoka welcomed participants' input about research opportunities and challenges related to this field that might help the NSRG identify the most appropriate next steps. She then introduced Dr. Peter Greenwald.

### **WELCOMING REMARKS**

Peter Greenwald, MD, DPH, Director, DCP, NCI welcomed participants to the workshop. He shared some of his experiences related to human evidence in order to illustrate the need for this workshop and for attention to basic science in energetics. Specifically, he mentioned that in 1980 there was a dispute among those working on dietary recommendations about the role of fat in the diet without enough basic science to support either side. There was a growing belief among those making dietary recommendations and epidemiologists that dietary fat was a big factor in breast and other

cancers. The approach taken then was to look at the percent of calories in the diet from fat and to adjust for total calorie intake. The message was good but people subsequently separated the idea of being fat vs. eating fat. Later, the Women's Health Initiative looked at dietary fat *vis a vis* breast cancer, but that study looked at percentage of calories of fat rather than grams of fat. Follow-up research is needed since that study did not show a statistical association between percentage of calories from fat and breast cancer. We now have examples, Dr. Greenwald said, where intervening earlier to prevent carcinogenesis is good but the effect may not be seen until about ten years later. There is much to address and basic science is a key component. In the behavioral area, the behavior of institutions needs review. City planners need to plan sidewalks so people can ride bicycles. Commercial interests need heightened awareness to translate the research findings to behaviors such as providing smaller glasses of soda at the movie theatres. Basic science to inform this picture is critical so the work of those present at this workshop is important.

John Milner, PhD, Chief, NSRG, DCP, NCI, next welcomed workshop participants. He provided additional background information to help set the tone for the workshop. He noted that diet is an important variable in the overall risk for cancer. Approximately 30% of cancers are related to diet and that number may increase since the incidence of obesity is now at about 65% of the population. Obesity has increased the risk across multiple cancers, but not for all cancers. Dr. Milner described the task ahead as one of explaining the relationship between energetics and an increased risk of cancer. There is a need to tease apart the pathways that explain energetics and increased risk of cancer. There is evidence that AMPK and mTOR can be influenced by eating behaviors. The scientific question becomes, "Are these energy or nutrient sensitive pathways?" There is evidence that some other factors can modify these pathways. Dr. Milner informed participants that there would be thought-provoking presentations at this workshop on tissue specificity and gene polymorphisms among other topics to spur research ideas on how to intervene and with whom to intervene to reduce the overall risk of cancer. He invited participants to discuss ideas presented. He mentioned that this workshop is the predecessor for a larger conference on issues related to nutrient versus energy sensitivities and which of these pathways should be singled out for further study to reduce the overall risk of cancer.

Dr. Sharon Ross served as the moderator for the first two speakers. Dr. Ross highlighted the biographical profile of the first speaker, Zhijun Luo, MD, PhD, Associate Professor in the Departments of Biochemistry at the Boston University School of Medicine, Boston, Massachusetts. Dr. Ross introduced Dr. Luo whose topic was the involvement of AMPK and related pathways in cancer prevention.

## **PRESENTATIONS**

### **I. Pathway: Involvement of AMPK and Related Pathways with Cancer Prevention**

Dr. Zhijun Luo informed participants that his presentation would focus on two aspects of AMPK: First, its role as a regulator of cellular metabolism and growth of cancer cells and second, its role in the regulation of Akt. AMPK can cause genetic changes that aid

the cell in making chronic adaptations such as the increase in mitochondrial biogenesis and enzyme expression in muscle associated with physical training. AMPK was first discussed in the scientific literature in 1972. It has been found in plants, yeast and mammalian cells and is a key regulator of metabolism. It is the kinase shown to be regulated by cellular energy state changes. It consists of 3 subunits:  $\alpha$ ,  $\beta$ , and  $\gamma$ . AMPK is activated by exercise, starvation and the fat-cell derived hormones adiponectin and leptin as well as by catecholamines and interleukin 6. Once activated, AMPK regulates downstream targets resulting in stimulating multiple events that enhance ATP generation as well as inhibiting others that consume ATP but are not necessary for acute cell survival. Enzymes inhibited by AMPK include mammalian homolog of Target Of Rapamycin (mTOR), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS) and glycerol phosphate acyltransferase (GPAT)—key regulators of protein, fatty acid and glycerolipid syntheses. AMPK is associated with the metabolic syndrome—a dysregulated metabolic state characterized in part by insulin resistance, a predisposition to Type 2 diabetes, hyperinsulinemia, predisposition to vascular disease (pro-inflammatory and pro-coagulant changes and premature atherosclerosis) and insulin resistance. The metabolic syndrome is often associated with inhibition of AMPK and increases in malonyl CoA. Activation of AMPK can prevent or correct alterations of signal events and energy metabolism caused by the metabolic syndrome.

Dr. Luo cited findings from epidemiological studies which support the link between AMPK and cancer. Some of the key points are as follows:

- Obesity increases the morbidity and mortality of many cancers
- Hyperlipidemia, hyperglycemia and hyperinsulinemia are risk factors for cancer
- Diabetic patients treated with metformin show a reduced incidence of cancer (25%)
- Patients with some cancers (breast and prostate), have a decreased plasma level of adiponectin

Experimental studies have found that for cancer induced by carcinogens or in tumor-prone animals exercise reduces the onset of cancer. Studies in animal models have shown that AMPK activators such as phenformin, metformin and AICAR inhibit tumor development. Oncogenic and tumor suppressor proteins that act as AMPK targets or activators include LKB1, TSC2, PTEN, FAS and p53. Dr. Luo explained the relationship between AMPK and each tumor suppressor protein. He singled out Fatty Acid Synthase (FAS), a metabolic oncogene found in many human cancers including breast, ovary and prostate. This may be a tumor therapeutic target.

Given this knowledge, Dr. Luo and his colleagues hypothesized that AMPK is linked with the metabolic syndrome and cancer. In the metabolic syndrome, the expression and secretion of IGF-1, with insulin and ROS among other factors can stimulate cell proliferation, hyperplasia and predispose the cell to mutagenesis. Dr. Luo and his colleagues tested their hypothesis that activated AMPK inhibits cell growth in experiments with AICAR which is an AMPK activator. Their investigations showed that AICAR inhibits the growth of prostate cancer cells, inhibits PC3 cells, acts differentially on LNCaP and normal PrEC, antagonizes the effect of androgen, down-regulates FAS and ACC and inhibits the stimulatory effect of androgen on mTOR. The A549 lung cells

which do not have LKB1 were resistant to AICAR treatment, however. In another study with PC3 cells to verify previous results, they used another AMPK activator, Rosiglitazone, and compared it to AICAR treatment. They measured levels of malonyl CoA and found both AMPK and Rosiglitazone reduced the level of malonyl CoA. In testing AICAR activity in LNCaP (human androgen-dependent prostate carcinoma cell line) and in normal PrEC (primary prostate epithelial cells as control) researchers found that primary prostate epithelial cells had decreased growth, probably due to: resistance to AICAR or due to the level of AMPK activation; primary prostate epithelial cells may adapt to higher levels of AMPK; or the cells may have suppressed AMPK activity at the molecular level. This occurred even though the standard activation of the two cell lines by AICAR is comparable. The effects of AICAR were mediated by AMPK. These investigations also showed that the stimulatory effects of androgen on FAS expression and on S6 kinase are suppressed when active AMPK is expressed. Dr. Lou and his colleagues also studied the regulation of gene expression by AMPK. Findings from these investigations led to the following conclusions:

- When AMPK is activated it causes inhibition of prostate cancer cell growth
- When AMPK is suppressed, cell proliferation is enhanced
- AMPK antagonizes the effect of active androgen receptor on mTOR, FAS and ACC--all necessary factors for accelerated growth of cancer cells.

Studies of suppression of AMPK and the result on cell proliferation showed significant increases in doubling time of many cell lines. In several investigations, Dr. Luo and his colleagues demonstrated that AMPK activation can suppress cell proliferation. In DN- $\alpha$ 1 (a dominant negative mutant of AMPK  $\alpha$ 1 subunit), the inhibitory effect of AICAR on expression of FAS was suppressed. To see if AMPK could inhibit cancer cells, Dr. Luo and colleagues used inhibition of tumor growth by metformin. After four weeks, they found that size of tumors in animals treated with metformin was less than in controls not treated with metformin. In a study of the role of AMPK in gene expression, Dr. Luo showed that many genes involved in cell proliferation, survival and tumorigenesis are regulated by AMPK.

Dr. Luo briefly described investigations of their hypothesis that AICAR enhances insulin activation of Akt. They found that Akt phosphorylation is enhanced by AICAR and that AMPK mediated the effect of AICAR on mTOR and enhanced Akt activation by EGF. However, they found no effect of AICAR on Akt in C4-2 prostate cancer cells. They hypothesize that AMPK may act on PTEN or on PI3K. However, to date, their research has shown that AMPK does not phosphorylate PTEN. This may be an area that warrants further study. Since the P13K-Akt signaling pathway is active in many cancers, AMPK's regulation of TSC2 and mTOR has important implications.

Dr. Luo concluded his presentation with the following comments:

- AMPK may function as a metabolic tumor suppressor
- Regulation of AMPK may impact cell proliferation and may provide a link between the metabolic syndrome and cancer.
- Paradoxically, AMPK activates Akt but regulates cell proliferation, perhaps via a global effect. More research is needed here.

- AMPK keeps protein and fatty acid synthesis in check in a normal energy state by regulating mTOR and FAS, so as to assure that the cell proliferation is under control. When cells confront energy shortage, AMPK activation production of more ATP and shuts down protein and fatty acid synthesis in order to save energy for ATP consuming processes required for acute cell survival. In contrast, when AMPK is persistently activated, it exerts a negative effect on cell growth. Collectively, AMPK can have both a preventive and therapeutic effect on the growth of malignant cells.

## QUESTIONS

### Question 1

*How does AMPK suppress androgen activity? Have you checked the phosphorylation of the androgen receptor?*

#### Response to Question 1

We don't know yet. We are currently checking this, but we don't know the answer yet. We did a microarray analysis and did not see changes in androgen receptors. We wanted to see if AMPK affected the ability of androgen to stimulate transcription but we did not see changes in androgen receptors. AMPK probably acts through translational control, as androgen can stimulate translation. We might check the phosphorylation of the androgen receptor. We might check if AMPK can affect the ability of androgen to stimulate transcription and see if they have opposite effects on transcription of mTOR and FAS and could look at it either at the pre or post translation level. This is probably the direction we will pursue.

### Question 2

*In speaking about neoplastic vs. normal cells, you showed some data with primary culture and the response was quite different between normal cells and neoplastic cells. Are you saying that AMPK regulation is more important for a neoplasm than it is for a normal cell?*

#### Response to Question 2

That is a really important question. We did not follow that. Some say in their data that activation of AMPK in primary cells caused cell senescence in primary cells. We did not see a significant change in cell numbers, but maybe the cell slowly dies or gets senile.

*That's the other part of the question. It looked like there was a subset that AMPK was lethal to and then it normalized. Is autophagy or are autophagic-type responses involved in what you're seeing in normal and in neoplastic cells?*

We did not check that. We should check that. That is a good question.

### Question 3

*AMPK is a sensor for low energy status. When you give AICAR with cells without low energy status, you are cranking up all these processes to deliver substrate. What happens to that substrate?*

#### Response to Question 3

Those substrates will be phosphorylated. However, we are not sure if the AICAR effect is solely mediated by AMPK because AICAR has effects independent of AMPK activation. In normal cells in the normal nutrient condition, when we treat cells with AICAR we definitely see the phosphorylation of AMPK substrate. Part of the effect of AICAR may be mediated by AMPK.

*What happens to the glucose uptake and to fatty acid synthesis?*

That's a good question. We did not check glucose uptake in cancer cells. Fatty acid synthesis is suppressed.

*So you are shutting down all these energy requirement processes?*

It's complex. For normal cells with normal response to free insulin, if we treat them with AICAR we don't see a significant change in glucose uptake, but we shut down or inhibit protein synthesis and fatty acid synthesis. If protein synthesis is shut down too long it definitely arrests cell growth.

#### **Question 4**

*Do you see increased fatty acid oxidation with these cells?*

#### **Response to Question 4**

It depends. It's hard to see fatty acid oxidation in these cells. In cancer cells, most of the energy source is from glycolysis. You can see it, but it's difficult.

#### **Question 5**

*You showed data from in vivo studies based on pharmacological intervention. Did you mimic that with dietary intervention or with metabolic state?*

#### **Response to Question 5**

No. That's the experiment done by Dr. Thompson.

#### **Question 7**

*Is there tissue-specific AMPK activation in diabetic patients?*

#### **Response to Question 7**

You mean pharmacologic for diabetic patients? No; that's why they use metformin.

#### **Question 8**

*You showed that AICAR can prevent the downstream pathways of mTOR-- phospho-S6 phosphorylation even though phospho Akt levels are going up. Do you think the activation of AMPK can subvert or inhibit the Akt pathway? Have you looked at other substrates of Akt to see if they are down regulated, as well?*

#### **Response to Question 8**

Yes, we found that the activation of AMPK uncoupled the Akt pathway. We have looked at pGSK3 and GSK3b. When we treated cells with AICAR, we consistently found that Akt phosphorylation was increased. In adipose tissue AMPK activation suppresses the glucose uptake by 3T3-L1 adipocytes.

#### **Question 9**

*Do you have any data on AMPK effects on TSC1 and TSC2?*

### **Response to Question 9**

That's a great question! I think AMPK only inhibits TSC2 but has no effect on TSC1. We carried out a kinase assay *in vitro* to see if mTOR is phosphorylated by AMPK. Although we saw mTOR phosphorylation, but we did not see a change in mTOR activity in terms of its activity toward 4E-BP. We believe mTOR's effect is through TSC2.

## **II. Regulation: Dietary Energy Intake, Exercise and Energy Sensing Pathways**

Henry J. Thompson, PhD, is Professor in the College of Agricultural Sciences and Director of the Cancer Prevention Laboratory at the Colorado State University in Fort Collins, Colorado. Dr. Thompson's research focuses on the relationship between antioxidant phytochemicals and on the mechanisms underlying the cancer inhibitory activity of energy restriction and exercise.

**Common alterations underlying the pathogenesis of chronic diseases.** Dr. Thompson opened his presentation with a slide showing the "common alterations underlying the pathogenesis of chronic diseases." He suggested that the audience think of cancer as one of four inter-related diseases: heart disease, obesity, Type 2 diabetes and cancer. At the core of these diseases are altered glucose utilization, cellular oxidation and inflammation as well as misregulation of cell proliferation, cell death (or apoptosis/autophagy) and vascularity; these are central effects of these disease states. There is rich literature in energy sensing related to the first three diseases (i.e. not in the cancer literature *per se*). Dr. Thompson and his colleagues study pre-malignant and malignant pathologies in the disease process in a rapid emergence model for breast cancer. Underlying this research is the notion that energy status/energy sensing regulates the carcinogenic process. Intracellular energy status differs in normal cells in different conditions of energy balance. Intracellular energy status also differs in normal vs. tumor epithelial cells (Warburg effect and variations beyond the Warburg effect). Intracellular energy status can limit the cell's ability to mount a response to external stimuli (i.e. via "E-messengers" and "E-sensors"). Energy availability affects transcriptional regulation and post transcriptional regulation. Physical activity as a function of its intensity, duration and frequency, affects energy balance and correspondingly, energy availability. Dr. Thompson outlined these basics and said that the amount of energy taken in and the amount of exercise impact internal sources of the fuel mixture that collectively determine cell energy availability and that varies within in the cell, noting that ATP is not always maintained at a constant level in the cell. Tissue specific responses to energy status may well play an important role in carcinogenesis but this possibility is not often discussed. Dr. Thompson has devoted much study to determining the effects of energy availability on post transcriptional regulation of protein function through phosphorylation (although acetylation is also important) with a primary focus on mTOR signaling and how this relates to the effects of energy availability on the processes of proliferation-vascularization-autophagy-apoptosis. These areas are key components in the investigation of energetics and cancer. Dr. Thompson next reviewed experimental data on this area.

**Planes of energy nutrition** Dr. Thompson discussed energy balance in terms of different planes of energy nutrition that are associated with different rates of growth and the maintenance of different stable body weight for individuals who have the same height. Lower planes of energy nutrition are associated with dose dependent effects on tumor incidence, multiplicity, latency, and mass. Protection against cancer is attributed to a decrease in cell proliferation, an increase in the pro-apoptotic signaling through the mitochondrial pathway, and a decrease in blood vessels around the tumors but not within the tumors. Data indicate that restricted energy intake also upregulates autophagy.

**AMPK-Akt-mTOR EB pathway or network** Dr. Thompson discussed research on the effects of energy balance on the AMPK-Akt-mTOR pathway or network in detail. The levels of pAMPK increased at lower planes of energy nutrition and signaling mediated via phospho activated Akt is decreased, with the predicted decreases in phospho mTOR and its downstream targets, 4E-BP1 and S6K being observed. These effects are consistent with observed effects on proliferation, apoptosis, autophagy and vascularization. There appears to be extensive cross talk among AMPK, Akt and perhaps mTOR. Effects on FoxO and sirtuins were also investigated and supports more detailed efforts to understand the “cross-talk” among the AMPK-AKT-mTOR network and pathways regulated by sirtuins and FoxO with a specific focus on their effects on autophagy, stress resistance, cell cycle arrest and cell death.

**Effects of drugs that perturb energy metabolism** The investigators next selected 2-deoxyglucose (2-DG) to study the effects of energy restriction on cancer. It is a glucose analogue which accumulates in tumor cells, increases AMP/ATP and blocks glycolysis. Dr. Thompson and his colleagues found that 2-DG at a dose an order of magnitude lower than doses used in aging research caused a reduction in the incidence of palpable cancers but had no effect on body weight gain and no effect on circulating factors in animals. They also looked at the effect of 2-DG on protein expression in mammary carcinomas. The findings were similar: a decrease in Akt; an increase in AMPK with effects on mTOR and the substrates that AMPK phosphorylates. In this rapid emergence model it is difficult to inhibit mammary carcinomas. Levels of 2-DG caused a profound reduction in incidence, multiplicity and size of mammary carcinomas.

Metformin is well known to activate AMPK. Dr. Thompson and his colleagues demonstrated the effect of metformin on the post-initiation phase of MNU-induced mammary carcinogenesis. In experimental animal (rats) data at levels of metformin that don't affect growth there was a dose-dependent reduction in multiplicity and a reduction in cancer incidence. They looked at phospho AMPK to see that AMPK activity is truly elevated—which it was. Rats with the same body weight but treated with metformin had decreased mTOR. Metformin can have an impact on carcinogenesis. Data showed that metformin can impact carcinogenesis at levels lower than those prescribed clinically to treat diabetes. In another experiment, Dr. Thompson and colleagues looked at Rapamycin to inhibit mTOR. They wanted a dose of Rapamycin that would not affect animal growth. A high dose does impact tumor mass (i.e. caused a reduction in tumor weight) but not tumor incidence or multiplicity. Rapamycin does not show the same selectivity as 2-DG or metformin. However, the Rapamycin data suggest that in

searching for the optimum point in the pathway to regulate, acting at the level of mTOR may not be ideal for the overall health of the organism.

### **Physical Activity**

Dr. Thompson and colleagues studied the effect of aerobic physical activity on body weight regulation and the carcinogenic response in the mammary gland in animals on non-motorized and motorized wheel running. Animals in the non-motorized wheel running group ran 7 days a week to a food reward. They ran from 90 minutes to about 4 hours a day. In this physically active group there was a modest reduction in final body weight and in the number of cancers/rat. Final body weight in rats in the restricted energy group showed a similar, modest reduction in final body weight and a somewhat higher number of cancers/rat than the physically active group. The sedentary control group had a higher final body weight and a higher number of cancers/rat than either the physically active or the restricted energy group. When the researchers switched to the motorized wheel running—40m/min, 7 days/week of self-selected duration for a food reward (not exercise to induce  $VO_2$  max) animals that ran at the standard or medium level of intensity were more protected than animals who ran any way they wished. Dr. Thompson hypothesizes that through effects on cellular pathways, exercise, like energy restriction, probably creates a pro-apoptotic environment. However, his research on physical activity and breast cancer found no impact on plasma estrogen levels. Animals in the exercised, mammary gland and mammary carcinoma groups all had activated AMPK. This is also seen in the liver and muscle. The biggest effect among the physically active animals is seen in the muscle which is not surprising. Exercise seems to have a greater impact on 4EBP1 than it does on S6K. Dr. Thompson postulated that this is the way to look at energetics and cancer. This is new data which is still being reviewed.

Physical activity studies focus primarily on aerobic activity. Another area for future study is effects of resistance training. There is a paucity of literature on resistance training. Resistance training is important in studying fuel mixture and energy availability. Dr. Thompson regards this area as a missed opportunity that needs to be addressed.

**Translational Opportunities** Dr. Thompson closed his presentation with some points regarding translational potential:

- Energy sensing pathways regulate the activity of multiple tumor suppressor genes such as LKB1, TSC2, PTEN, FoxO, p53, Rb and perhaps others and this may explain, at least in part, their power to prevent carcinogenesis. We could look at energy sensing as a way to re-regulate the activity of these genes.
- Energy sensors may be targeted for cancer prevention by drugs such as metformin, 2-DG (this preferentially localizes in developing transformed cells but there are some problems with 2-DG) and Rapamycin (may be too low down in the pathway)
- A dieting paradigm to selectively delete transformed cells through modulation of autophagy, Warburg effect and the FoxO-Sirtuin relationship appears of value to investigate. The hypothesis is that if you inhibit autophagy in the energy-restricted context, you will delete transformed cells by apoptosis

- Energy sensors such as activated AMPK may provide a biomarker of an “energy protected state” clinically and serve as health indicators. This is a translational idea.

## **Questions for Dr. Thompson**

### **Question 1**

*Was the mTOR pathway activated in mammary induced carcinoma? How did metformin reduce cancer? Did you investigate metformin’s effect on proliferation?*

### **Response to Question 1**

The mTOR pathway is activated in carcinomas. The metformin data are very recent. We are still reviewing the metformin data.

### **Question 2**

*In terms of circulating AMPK: Did you measure plasma? How much variability is there in circulating concentrations?*

### **Response to Question 2**

We think the place to look at AMP kinase would be in circulating lymphocytes. It’s problematic to measure in rats. We are doing a weight loss intervention in breast cancer survivors –physical activity and energy restriction—and it makes more sense to measure it in those ladies. We would have more blood to work with and we could work on methodologies. We are asking what the predisposing factor is in breast cancer. Is it the fat among the obese or is it energetics? We think the fat distracts us from looking at the role of energetics. It is important for a woman going on a diet to know this is a journey, not a destination. Our hypothesis is that the women don’t have to lose all the weight before they are protected against cancer. We hypothesize that they are protected from cancer from the moment they begin the journey.

### **Question 3**

*In the clinic, those with cancer or with calorie restriction complain of fatigue. How do energy sensing pathways affect the perception of fatigue?*

### **Response to Question 3**

These energy sensing pathways are clearly operative in various compartments of the brain. If we knew these pathways better, we could understand fatigue better. However, in terms of the concept of energy restriction, everyone thinks you have to be losing weight and be on a diet. This research is about adopting a lifestyle that has less energy available in the system everyday but it does not consist of constant dieting. When people are large they have to think in terms of dieting getting rid of cancer cells. The work is a model from all the different planes of energy nutrition. The journey to get to a lower BMI may involve some fatigue, but this is not a starvation model.

### **Question 4**

*Some fatigue may be at a later stage. Cachexia is just one example. We at the National Institute of Aging are interested in fatigue at a clinical level. We want to understand the mechanisms of fatigue.*

### **Response to Question 4**

The CR group of people who follow a severe calorie restricted diet are actually at increased risk for several diseases since they are following an extreme. There is good mortality data showing you don't want to be too thin.

### **Question 5**

*Exercise activates AMPK and causes hypertrophy. You also need protein synthesis. Have you measured AMPK phosphorylation and mTOR pathways during different periods of exercise and compared that with cancer cells?*

### **Response to Question 5**

There are so many interesting experiments to do such as acute vs. chronic effects of physical activity; should you look in muscle and when, etc. How those questions fit into the puzzle with cancer I don't know. Physical activity is harder to study than energy restriction. We struggle deciding which things are right to do first. First, we need to show conditions that inhibit carcinogenesis. In terms of energy sensing, when you exercise less you are better protected than when you exercise more; this is a fascinating question to study. Women who are smaller and exercise less are better protected against cancer than larger women who exercise more and we don't know why. We know physical activity is important but there are still many unanswered questions. We might measure the ratio of protein synthesis to autophagy but we have not done that yet.

### **Question 6**

*What is going on with the way glucose is being produced and utilized in the tumor? Is it the Warburg effect?*

### **Response to Question 6**

There are two arguments right now best articulated by Craig Thompson in terms of whether or not the survival that's being induced in the tumor—the autophagic response—is actually supporting the ability to kill cells or blocking. I wish I knew more. We have not measured the level of protein synthesis to autophagy.

### **Question 7**

*What makes people more susceptible? Is it mutations of pathways?*

### **Response to Question 7**

Every mutation shown enhances carcinogenesis—the LKB1, TSC2, p53, PTEN, etc. They are all very relevant suppressors and if you can achieve re-regulation and suppression through energy availability that would be an important observation. Not too many people are talking about that yet. There are at least five recognized suppressors in pathways of energy sensing networks and it's bigger than I have shown today. This is a hot topic in the therapeutic context now typically regarding the Warburg concept.

### **Question 8**

*Can we go back to the AMP/ATP ratios to get important concepts?*

### **Response to Question 8**

Yes. We have more sensitive methodologies now to look at that ratio than we did 30 years ago when it was first done. However, we all argue about ATP data; it is controversial. No one argues about AMPK.

### **III. Use of Animal Models for Examining Cellular Energetics and Diet Interrelationships**

Charles Vinson, PhD, is the Section Chief in Laboratory Metabolism at the National Cancer Institute. Dr. Vinson opened his presentation with a graphic depicting the growth in the size of man from the early ape-stage to the present, obese human figure. It is known that obesity is associated with cancer but research is needed to understand why certain tissues seem to be more susceptible to cancer in obese humans than other tissues. Restricting calories has shown profound effects in many tissues and genetic models. Restricting food intake causes a significant decrease in the number of tumors that occur. A study of the effect of 30% calorie restriction on spontaneous tumors in p53<sup>-/-</sup> and p53<sup>+/+</sup> mice resulted in increased survival and correlated with a higher level of IGF-1.

Researchers utilize genetic tumors and chemically-induced tumors in their investigations. It is easier to see the effects of calorie restriction in genetic tumors. In looking at growth factor levels and Wnt-1 TG mammary tumor growth in lean, overweight and obese mice that were ovariectomized, the increased tumor burden in the obese mice appears to correlate with hormones. Dr. Vinson stated that the adipocyte secretes hormones such as adiponectin, resistin and leptin. To investigate the relationship of adipokines in obesity to cancer, Dr. Vinson described studies using the A-ZIP/F-1 “fatless” mice developed in his laboratory. This mouse eats twice as much as normal and develops high insulin levels and diabetes. It has no adipokines but it has diabetes. The livers are twice the size of the normal liver and almost identical to those seen in obese mice. Glucose is elevated two fold in males and females and insulin levels are 500 fold higher. Free fatty acids are elevated two fold. Sixty percent of the male fatless mice die at 30 weeks; they are classic diabetic mice. Diabetes in these mice was reversed by transplanting fat back from wild type mice. The absence of fat drives this prototype.

Dr. Vinson and his colleagues hypothesized that since these mice have no fat they would be resistant to cancer. In a two-stage skin carcinogenesis study, they found that these mice have increased susceptibility. On the A-ZIP/F-1 mice the papillomas arise quickly which raises questions about whether the diabetes potentiates the cancer in these animals since these mice have no serum adipokines but they have increased insulin and growth hormone. It is difficult to get these mice to live past 20 weeks; they get too sick.

In a study of the C3(1)/SV40 T antigen transgenic breast tumor in these mice, the A-ZIP/F-1 mice did not accelerate growth of breast tumors. The researchers crossed these mice to a second breast tumor in the MMTV-Polyoma Middle T (PyMT) and got no result. Future research might cross these mice to different genetic tumors and see which ones are affected and which are not. Some tumors are energy independent and others are not.

In these mice the adipokines are missing, insulin and growth hormone are higher and IGF-1 is higher as are the inflammatory cytokines. The skin in these mice shows activated receptor tyrosine kinase pathways (Akt and ERK). Future research might look at caloric load and resulting insulin resistance vs. inflammatory response and increased

cancer risk to ascertain which pathway is the critical one for cancer prevention. These studies have shown that these mice with no adipokines still get potentiation of tumors so those adipokines are not critical or essential. Dr. Vinson closed his presentation saying that the important area to pursue seems to be in solving whether insulin resistance or inflammation is potentiating the increase in cancer risk.

## **QUESTIONS**

### **Question 1**

*Could you give an anti-inflammatory drug to eliminate potentiation in your investigations?*

### **Response to Question 1**

We could and that would decrease papillomas in the wild type mice as well as in the fatless mice. It is hard to find a way to study this.

### **Question 2**

*When you cross the fatless mice with others do you get an intermediate level of fat?*

### **Response to Question 2**

The fat is dominant. These mice have absolutely no fat. The line has been stable as a genetic entity for over ten years.

### **Question 3**

*When you transplant fat is the insulin resistance eliminated and is the inflammatory response also eliminated?*

### **Response to Question 3**

No. We have not looked at the inflammatory response per se. The more fat you transplant the more you resist the diabetes so it seems to be the quantity of fat that causes the diabetes. Technically, we could not transplant the fat into the abdominal area of these mice due either to lower vascularization or some other technical issue so we can't answer the question of abdominal vs. peripheral fat location.

### **Question 4**

*Is there a dose dependence of calorie restriction vs. tumor formation?*

### **Response to Question 4**

The more you do the less tumor formation there is but you can't restrict calories too much or the animal dies. You can't restrict more than 40% and you don't need to go further than that amount; you get into other issues if you restrict more than that percentage.

### **Question 5**

*Does the rate of tumor formation keep going down as the calorie restrictions go up?*

### **Response to Question 5**

Yes, calorie restriction is effective up to 40% calorie restriction. If you go too far, the animal dies.

### **Question 6**

*Have you done calorie restriction in the fatless mice?*

**Response to Question 6**

They died. They need to eat twice as much as normal mice. If you restrict calories they go into a hibernation state and die. They have no food stores.

**Question 7**

*If you dissect that part of the pathway could you cross with a mutant for NRF2?*

**Response to Question 7**

You affect the wild type mouse and the A-ZIP mouse so it's hard to evaluate but that's really the only way to go.

**Question 8**

*Several laboratories have used an animal insulin resistance model to destroy the islets in the pancreas then give insulin to maintain normal levels.*

**Response to Question 8**

We could get rid of the islets so the insulin levels would go down but the thing to do is to break this inflammation genetically and see how that affects tumor formation, e.g. papilloma formation, and be aware that what you do will affect both the wild type and the A-ZIP mice.

**Question 9**

*Have you given these mice metformin? If the drug does not affect insulin levels but it decreases tumors that would tell you something about the increase in energy uptake. And maybe just decreasing the amount of food intake would show something, i.e. it's not the level you are at but how much you are taking in.*

**Response to Question 9**

I think we have given them metformin. They don't respond to most anti-diabetic drugs. The problem is these mice are so sick you have to keep feeding them all the time. They have to have a pellet every 4 hours or so, but that's a good experiment. The problem with carcinogenesis experiments is that they are cumbersome. It would be nice if it were at the genetic level. It would be good to see if we got a dramatic effect with the Wnt-1 mice then we would have a better experimental window to see how interventions could affect the animals.

**Question 10**

*Do these mice cycle? If looking at obesity and estrogen this might be a good model to tease out those dynamics. This may be an interesting model for teasing out some dynamics such as estrogen and obesity in cancer.*

**Response to Question 10**

The females get pregnant but they can't lactate. Their estrogen levels are normal.

**NEXT STEPS**

Dr. John Milner thanked Dr. Tsuboyama-Kasaoka and Dr. Ross for organizing an outstanding meeting. Dr. Milner pointed out some of the highlights of today's meeting: energetics and how that relates to the cancer process; AMPK and how it relates to many signaling pathways; how AMPK relates to overall energy intake and dissipation of that energy through exercise and some models. He then solicited topic areas for an expanded

workshop to be held possibly in about eight months. Possible future research areas would include cross talk and autophagy. Workshop participants recommended the following topic areas for a future expanded workshop:

- Energetic components
  - Starving animals have smaller offspring
  - Energy sensitive pathways: It appears that limiting energy availability affects the conversion process from pre-malignancy to malignancy and we don't know why; that might be important.
  - It is not only calories that are important but the energy sensitive pathways and the nutrient sensitive pathways; we may need to join these two pathways together.
  - food intake regulation
- Histone deacetylase/Pathway regulation
  - Is it a combination of phytochemicals with fat that might regulate these pathways?
- Study models with negative results
  - Investigate why is there sensitivity in some organ sites and not in others and juxtapose that model with the energy sensitive model.
- Autophagy
  - It can be protective or stimulatory in terms of cell death
- Circulating hormones—look at those rather than focusing on BMI
  - See if there is a correlation between reduction in circulating hormones or metabolites due to exercise or energetics and reduction in cancer. Perhaps study these components rather than focusing on BMI.
  - Just because a cancer has an increased risk with obesity does not mean that those cancers that do not have that increased risk will not also be sensitive to things that decrease energy to the tumor, e.g. lung cancer which does not have a significant obesity component. If you decrease energy you may also decrease tumor formation. There is some evidence of exercise and a reduction in lung cancer.
- Resources: money, personnel and collaboration are issues to access relevant genetic models
  - Need to identify people in key topic areas
  - Identify specific topics to study such as biomarkers and cross talk and study those in animal models
  - Study new genetic models to see what influences the pathways
- Fatigue
  - Role of diet in minimizing toxicity of cancer treatment
  - Lethargy; ability to tolerate chemotherapy vs. not able to tolerate
  - Study negative energy balance/cachexia in patients. If their energy state is increased would that also increase their cancer? Study the obese that develop cancer then lose weight and see if that is protective. This relates to studying normal vs. neoplastic cells; see how long the neoplastic state lasts.
- Biomarkers
  - Look at AMPK as a biomarker or look at other energy sensitive markers such as ATP or ratio of ATP/AMP or others?
  - Do one session on bioenergetics and specific tumors; what is the cross talk between the amount of energy and a cancer cell within an organism?

- Nutrient genomic activities and exercise activities
  - Explain why there are gene-nutrient interactions and how nutrients affect DNA repair and other processes
  - Look at the strength of the evidence about certain foods and their impact on cancer prevention; look at how to integrate those with the total diet
  - Role and amount of exercise vs. free radical generation
  - Look at myokines; how much carbohydrate vs. fat goes into mitochondrial fluxing; look at modest exposures vs. the extreme
  - Nrf2: several things stimulate Nrf2 and these might be important modifiers. We may have to think not just in terms of calories but in terms of energy sensitive pathways and nutrient sensitive pathways. Maybe we can join those two pathways together.
- Timing or stage of development
  - Obesity is an issue in children now; perhaps relates to epigenetics
  - Should we investigate best time to modify the link between obesity and cancer? How strong is the evidence?
  - There are no good models of adolescent obesity in rodents. However, Dr. Vinson has a mouse that is born without fat but develops fat within about one month. It begins life with a large, white, fatty liver but then gets well. It would be possible to study the residual impact of fat in that animal model. The fat-1 mouse makes its own omega-3 fatty acids which changes inflammatory processes. That mouse might be linked to Dr. Vinson's fatless mouse.

## CONCLUSION

Dr. Sharon Ross concluded the workshop with a brief summary of the presentations. AMPK is in a number of processes. It is an energy sensor that may be implicated in cancer particularly via its effects on mTOR. Studying energy sensitive pathways is complex as are eating and physical activity behaviors and the involvement of myokines. Further research is needed in these areas.

Animal models are useful to tease out important components. A possible approach would be to mix different animal models. Biomarkers are also needed to move cellular energetics and cancer prevention forward; more precise measures of energy intake and exposure are needed. Susceptibility factors may vary among the population in energy pathways and these, too, require further investigation. One possible paradigm to pursue in order to identify key molecular targets might be to study energy intake and a specific molecular target such as mTOR and see how that is up-regulated under a scenario of excess energy; the outcome might be cellular growth.

Dr. Ross thanked all attendees for their participation. She invited all to review the handouts on AMPK and to please contact her and her colleagues at the NCI with any questions, comments or suggestions. They hope to hold a larger workshop on this topic in several months and possibly sponsor an initiative (such as a Program Announcement or a Request for Proposals) for research in this area. A paper summarizing this mini-workshop will be posted on the Web in the near future.