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Higher Concentrations of Glucose or Insulin Increase the Risk of Various Types of Cancer in Obesity or Type 2 Diabetes by Decreasing the Expression of p27Kip1, a Cell Cycle Repressor Protein

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Abstract

Research Aims: Obesity and type 2 diabetes are known to be associated with increased risk of various types of cancer. However, the molecular biological mechanism of how the risk of cancer is increased in obesity or type 2 diabetes is not known. The aim this research is to investigate if the decreased expression of p27Kip1, a cell cycle repressor protein, plays a central role in this mechanism. **Research Methods, Previous Studies and Theoretical Backgrounds:** It is well known that the expression of p27Kip1 is increased by numerous nutritional or chemopreventive anti-cancer agents. But it has never been known that the expression of p27Kip1 could be decreased, rather than increased, and the risk of cancer could be increased, rather than decreased. This problem was solved recently and this new analytical method was used in this study. **Results:** 1) The expression of p27Kip1 was indeed significantly decreased in human obese type 2 diabetic individuals relative to the lean normal controls. 2) The expression of p27Kip1 was also significantly decreased in genetically obese rodents relative to the lean normal controls. Additionally, in obese rodents, the concentrations of glucose or insulin were significantly increased relative to the lean normal controls. 3) Using human cells cultured *in vitro* it was found that the increased concentrations of glucose or insulin decrease the expression of p27Kip1. **Conclusions:** These results suggest that higher concentrations of glucose or insulin increase the risk of various types of cancer in obesity or type 2 diabetes by decreasing the expression of p27Kip1.

Keywords

Obesity, Type 2 Diabetes, Cancer, p27Kip1 mRNA, Translation Initiation, 5'-Untranslated Region (5'UTR), 5'-End Cap of p27Kip1 mRNA, Upstream Open Reading Frame (uORF), Internal Ribosome Entry Site (IRES)

1. Introduction: Obesity and Type 2 Diabetes Are Associated with Increased Risk of Cancer

"Obesity is on its way to replacing tobacco as the number one preventable cause of cancer. We need to confront this growing problem and develop all the necessary tools to limit its impact."—Clifford Hudis, MD, 2013-2014 ASCO President [1] [2].

According to research from the American Cancer Society, excess body weight is thought to be responsible for about 8% of all cancers in the United States, as well as about 7% of all cancer deaths [3]. Being overweight or obese is clearly linked with an increased risk of many types of cancer, including cancers of the breast (in women past menopause), colon and rectum, endometrium (lining of the uterus), esophagus, kidney and pancreas [3]. Being overweight or obese might also raise the risk of other cancers, such as: gallbladder, liver, non-Hodgkin lymphoma, multiple myeloma, cervix, ovary and aggressive forms of prostate cancer [3].

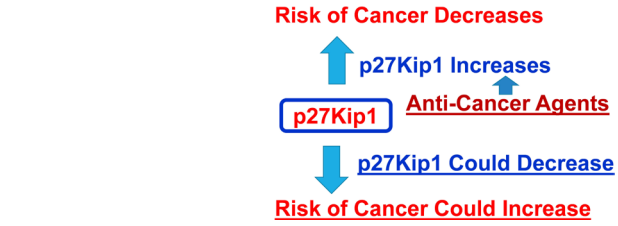
As for the type 2 diabetes, the American Diabetes Association states that researchers are continuing to explore the link between type 2 diabetes and certain cancers, including: liver, pancreas, uterus, colon and bladder [4].

Type 2 diabetes and certain cancers share some risk factors, from age, gender, and ethnicity to lifestyle factors like inactivity, smoking, and alcohol [4] [5]. The good news is that some of these risk factors are within your control to manage [4] [5]. In 2010, the American Diabetes Association and American Cancer Society jointly issued a consensus report on type 2 diabetes and cancer [6]. It stated in part that: "Type 2 diabetes and cancer share many risk factors, but potential biologic links between the two diseases are incompletely understood".

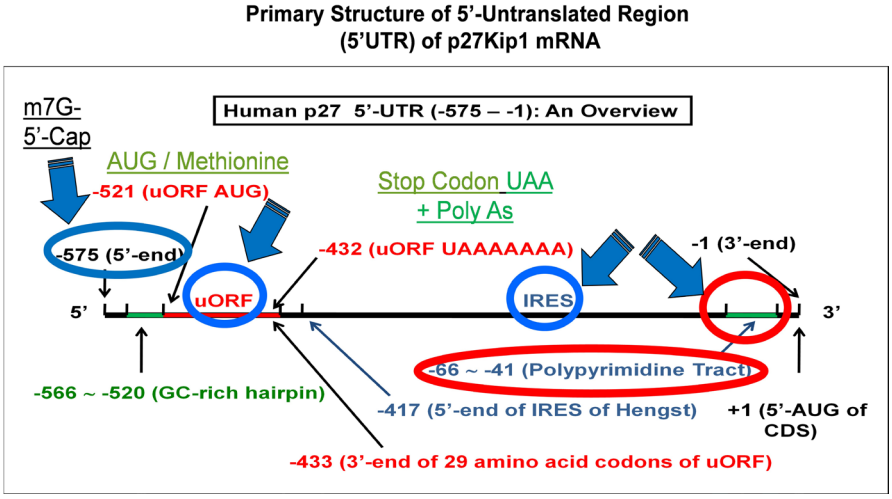
2. Methods, Previous Studies and Theoretical Backgrounds: p27Kip1, a Cell Cycle Repressor Protein, Could Either Decrease or Increase the Risk of Cancer (Figure 1(a) and Figure 1(b))

2.1. p27Kip1, a Cell Cycle Repressor Protein, Decreases the Risk of Cancer

Numerous nutritional and chemopreventive anti-cancer agents decrease the risk of cancer by increasing the expression of p27Kip1, a cell cycle-repressor protein. When increased, p27Kip1 inhibits cell cycle transition from G1 to S phase, DNA replication in the S phase and cell division in the M phase [6] [7]. It is known



(a)



(b)

Figure 1. p27Kip1, a cell cycle repressor protein, could either decrease or increase the risk of cancer.

that anti-cancer agents specifically-regulate the expression of p27Kip1 without directly affecting the expression of other G1-to-S phase cell cycle regulatory proteins including p21 (Cip1Waf1) [7] [8].

Anti-cancer agents increase the expression of p27Kip1 protein primarily at the level of translation, not at the level of transcription [6] [7] [8]. Unlike expression of any other cell cycle regulatory proteins, expression of p27Kip1 protein is very unusual [6] [7] [8]. The mRNA of p27Kip1 has a very long and unusual 5'-untranslated region (5'UTR) (from −575 to −1 in human) [7] [8] [9].

Anti-cancer agents increase the expression of p27Kip1 by a so-called cap-independent translation initiation mechanism using two elements in the 5'-untranslated region (5'UTR) of p27Kip1 mRNA [9]. First (a) 5'-end cap located at the position −575 in the 5'UTR will always be compromised by the metabolites of anti-cancer agents [9]. Second (b) internal ribosome entry site (IRES) that is centered on the polypyrimidine tract located from −66 to −41 [9]. Internal ribosome entry site attracts and binds 40S ribosomes for translation initiation of p27Kip1 [9].

2.2. Recent Evidence Suggests That the Decreased Expression of p27Kip1 Could Increase, Rather than Decrease, the Risk of Cancer in Certain Cases

There is an additional element in the 5'-untranslated region (5'UTR) of p27Kip1

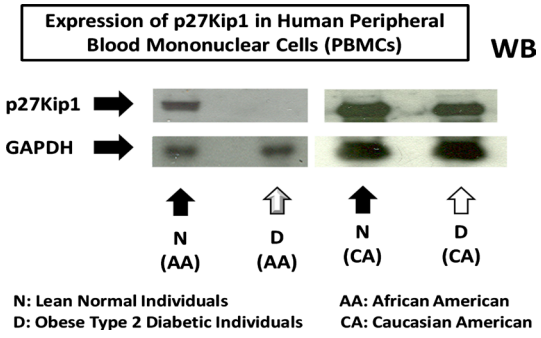
mRNA [9]. This element is called upstream open reading frame (uORF) [10]. Many human mRNA transcripts including p27Kip1 mRNA contain at least one upstream translational initiation site that precedes the main coding sequence (CDS) and gives rise to an upstream open reading frame (uORF) [10]. uORF of p27Kip1 mRNA contains AUG codon on the extreme 5'-side beginning at −521 and UAAAAAAAA on the extreme 3'-side beginning at −432 [9]. In between these sequences, it contains codons for 29 amino acid residues including AUG [9].

The experiments focused on the functional analysis of AUG-initiated uORFs mostly demonstrated inhibitory effects on downstream translation [10]. Thus, any cancer agents going through the uORF would decrease, rather than increase, the expression of p27Kip1 protein and thus increase, rather than decrease, the risk of cancer [9]. Tionhese cancer agents going through uORF in the 5'-untranslated region (5'UTR) of the p27Kip1 mRNA will be called "pro-cancer agents" rather than "anti-cancer agents" [9].

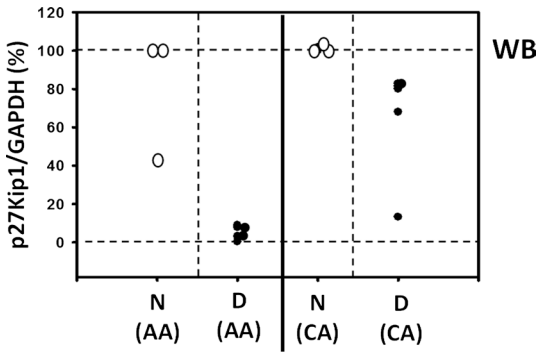
Sections 3-5 below describe results: The results are described of the decreased expression of p27Kip1 in human models of obesity or type 2 diabetes (in Section 3), rodent models of obesity or type 2 diabetes (in Section 4) and in *in vitro* human cell culture systems (in Section 5).

3. Expression of p27Kip1 Decreases in Human Models of Obesity and/or Type 2 Diabetes (Figures 2(a)-(c))

Expression of p27Kip1 indeed decreases in human models of obesity and/or type 2 diabetes [11]. Expressions of p27Kip1 in human peripheral blood mononuclear cells (PBMCs) are significantly and in certain cases severely lower in obese type 2



(a)



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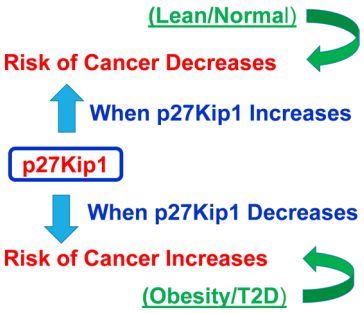


(c)

Figure 2. p27Kip1 decreases in human models of obesity and/or type 2 diabetes.

diabetic individuals relative to lean normal controls [11]. The decrease is severe—by over 95%—in obese type 2 diabetic African Americans relative to lean normal African Americans [11]. By contrast, the decrease is not that severe—by 20% to 80%—in Caucasian Americans [11]. The decrease appears to be gender neutral.

The American Diabetes Association released a statement that the "estimated 100 million Americans at risk for or with diabetes are also at increased risk of several types of cancer" [5]. Additionally, the consensus report issued jointly by the American Diabetes Association and American Cancer Society stated that in "the US, African American are more likely to develop and die from cancer than other race or ethnic groups" and that in "the US, type 2 diabetes and its complications disproportionately affect a number of specific populations including African Americans compared with non-Hispanic whites and while incompletely understood, genetic, socioeconomic, lifestyle, and other environmental factors are thought to contribute to these disparities" [6].

4. Expression of p27Kip1 Decreases and the Serum Levels of Glucose or Insulin Increase in Rodent Models of Obesity and/or Type 2 Diabetes (Figures 3(a)-(c))

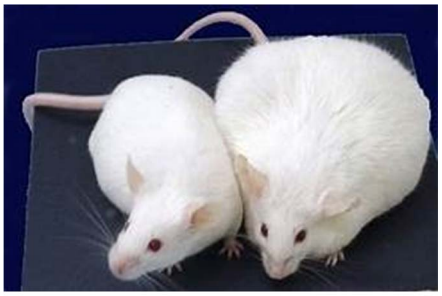
It was reported in 2005 that obesity promotes 7,12-dimethylbenz(a)anthracene-induced mammary tumor development in female Zucker rats [12].

Expression of p27Kip1 protein decreases in rodent models of obesity and/or type 2 diabetes [13]. The hepatic expression of p27Kip1 in homozygous leptin receptor-deficient obese Zucker rats decreased significantly by about 19% relative to heterozygous lean normal controls [13].

In contrast to the expression of p27Kip1 protein in the liver, the serum levels of glucose or insulin increased significantly in obese Zucker rats by about 33% and 2500%—this is not a numerical typo—respectively, relative to the lean normal controls [13].

Almost identical results were obtained using homozygous leptin-deficient obese ob/ob mice relative to heterozygous lean normal controls [13].

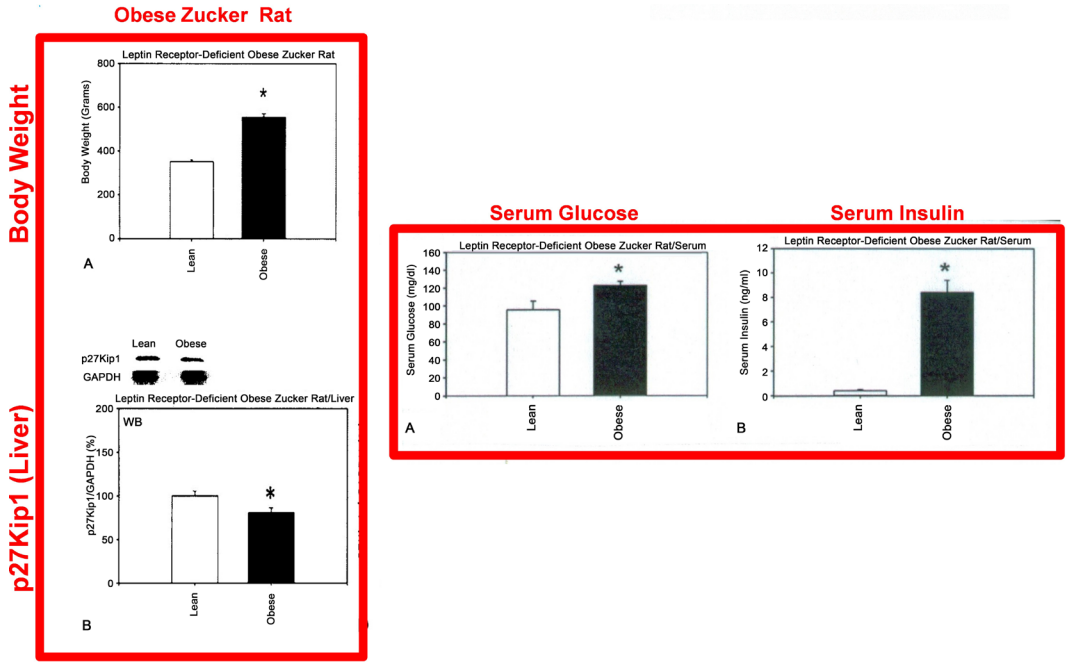
All experimental results obtained using the rodent models of obesity and/or type 2 diabetes suggested that glucose/insulin were "pro-cancer agents".



Zucker Lean Control Rat (left)

Zucker Obese Rat (right)

(a)



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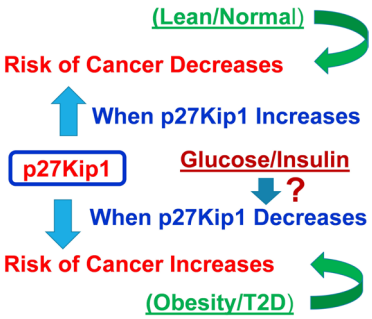
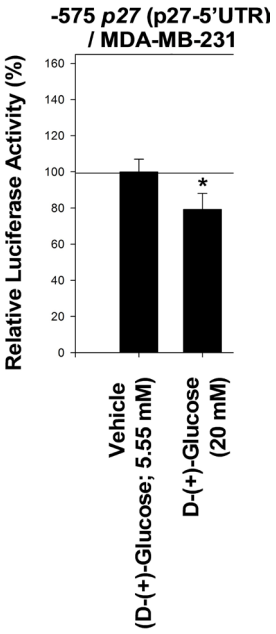


(c)

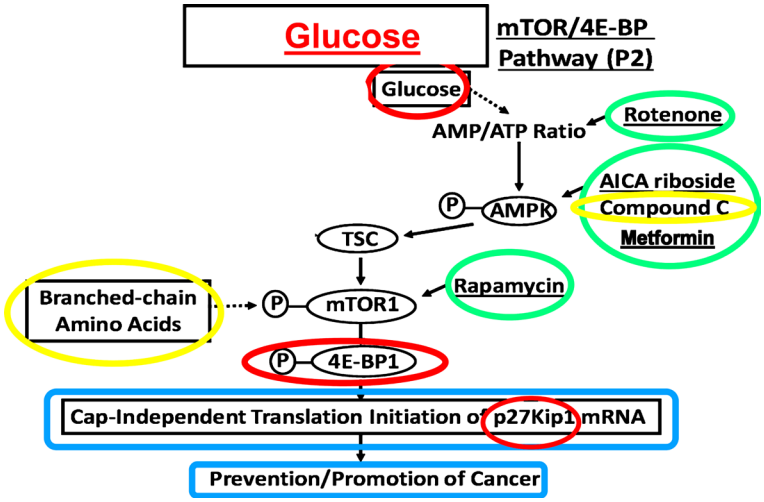
Figure 3. p27Kip1 decreases and glucose/insulin increases in rodent models of obesity and/or type 2 diabetes.

5. Increased Concentrations of Glucose or Insulin Decrease the Expression of p27Kip1 in Cultured Human Cells *in Vitro* (Figures 4(a)-(d))

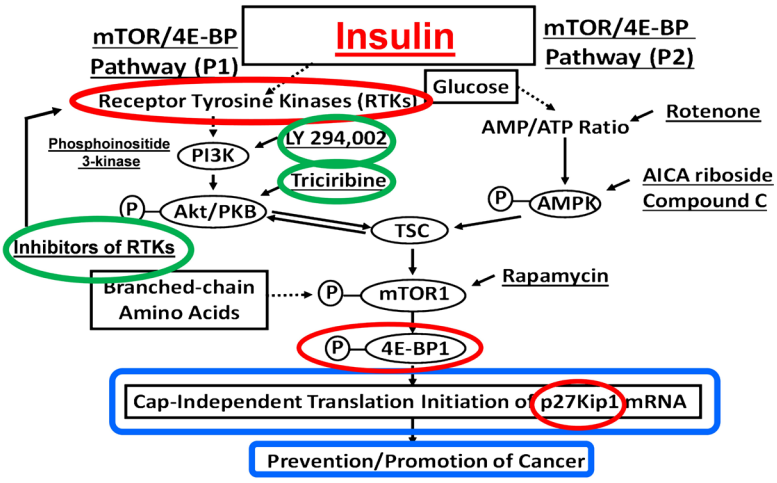
To investigate if there is a cause-effect relationship between the amount of


-575 p27 (p27-5'UTR)
/ MDA-MB-231
Relative Luciferase Activity (%)
160
140
120
100
80
60
40
20
0
*
Vehicle
(D-(+)-Glucose; 5.55 mM)
D-(+)-Glucose
(20 mM)

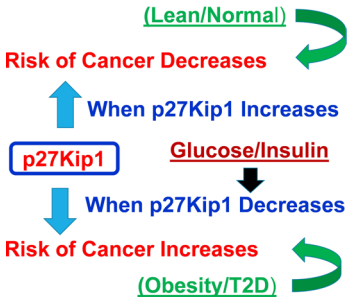
(a)


Glucose
mTOR/4E-BP
Pathway (P2)
Glucose
AMP/ATP Ratio
Rotenone
AICA riboside
Compound C
Metformin
P
AMPK
TSC
Rapamycin
Branched-chain
Amino Acids
P
mTOR1
P
4E-BP1
Cap-Independent Translation Initiation of p27Kip1 mRNA
Prevention/Promotion of Cancer

(b)


mTOR/4E-BP
Pathway (P1)
Insulin
mTOR/4E-BP
Pathway (P2)
Receptor Tyrosine Kinases (RTKs)
Glucose
AMP/ATP Ratio
Rotenone
Phosphoinositide
3-kinase
PI3K
LY 294,002
Triciribine
AICA riboside
Compound C
P
Akt/PKB
P
AMPK
TSC
Inhibitors of RTKs
Branched-chain
Amino Acids
P
mTOR1
Rapamycin
P
4E-BP1
Cap-Independent Translation Initiation of p27Kip1 mRNA
Prevention/Promotion of Cancer

(c)



(d)

Figure 4. Increased concentration of glucose/insulin decreases the expression of p27Kip1.

glucose or insulin and the rate of the translation initiation of p27Kip1 protein, luciferase reporter plasmid of the DNA sequence of the 5'-untranslated region (5'UTR) of the p27Kip1 mRNA was transfected into the cultured human cells *in vitro*.

5.1. Increased Concentrations of Glucose Decrease the Expression of p27Kip1 in Human Cells Cultured *in Vitro* (Figure 4(a), Figure 4(b) and Figure 4(d))

The results indicated that the rate of the translation initiation of p27Kip1 protein decreased significantly when the normal concentration (5.5 mM) of glucose in the cell culture medium was increased to higher concentration (20 mM) [7]. If glucose were an anti-cancer agent, increased concentration of glucose should have increased the expression of p27Kip1. Thus, this observation has suggested that glucose is one of the "pro-cancer agents".

Subsequently, the potential metabolic pathway between increased concentration of glucose and decreased expression of p27Kip1 was then investigated using the same 5'-UTR-luciferase reporter plasmid, but adding various inhibitors or stimulators of the potential pathway in between [7] [8].

The results suggested that the potential metabolic pathway from glucose to p27Kip1 could be divided into two consecutive segments, namely (a) the earlier segment that begins with glucose and ends with the tuberous sclerosis complex (TSC) proteins and (b) the later segment that begins with the tuberous sclerosis complex (TSC) proteins and ends with p27Kip1. The earlier segment is involved primarily in the energy metabolism including mitochondrial electron transfer chain, AMP/ATP ratio, and 5' AMP-activated protein kinase (AMPK). The later segment is involved primarily in the cap-dependent translation initiation mechanism of the 5'-untranslated region (5'UTR) of the p27Kip1 mRNA including mammalian target of rapamycin (mTOR) complex 1 and eukaryotic translation initiation factor 4E binding protein (4EBP1) [14].

5.2. Increased Concentrations of Insulin Also Decrease the Expression of p27Kip1 in Human Cells Cultured *in Vitro* (Figure 4(c) and Figure 4(d))

The potential metabolic pathway between increased concentrations of insulin and decreased expression of p27Kip1 was also investigated using the same

5'-UTR-luciferase reporter plasmid, but adding various inhibitors or stimulators of the potential pathway in between [7] [8].

The results suggested that the potential metabolic pathway from insulin to p27Kip1 could be divided into two consecutive segments as well, namely (a) the earlier segment that begins with insulin and ends with the tuberous sclerosis complex (TSC) proteins and (b) the later segment that begins with the tuberous sclerosis complex (TSC) proteins and ends with p27Kip1.

The earlier segment is involved primarily in the receptor tyrosine kinase metabolism including insulin receptors, phosphoinositide 3-kinase (PI3K) and Akt/PKB. The later segment is the same cap-dependent translation initiation mechanism as described above for glucose.

Summary and Analysis of the Findings Presented in the Results Sections 3-5 Above:

Results Section 3 above: African Americans have been known to be more susceptible to the risk of cancer due to obesity or type 2 diabetes relative to the Caucasian Americans. The extremely decreased expression of p27Kip1 in the African Americans is consistent with these historical and epidemiological observations.

Results Section 4 Above: Genetically obese or type 2 diabetic rodents are known to be more susceptible to the risk of cancer. The significantly decreased expression of p27Kip1 in the obese or type 2 diabetic rodents is also consistent with the experimental observations reported in recent years. Additionally, we observed in this study that the levels of glucose or insulin were significantly elevated in the genetically obese rodents.

Results Section 5 Above: Higher concentration of glucose or insulin decreases the expression of p27Kip1 in *in vitro* human cell culture system. Further investigations into the molecular biological mechanisms revealed that there is a consistent and specific pathway where glucose or insulin decreases the expression of p27Kip1.

6. Conclusion

Obesity and type 2 diabetes are associated with increased risk of cancer, but the links between obesity, type 2 diabetes and cancer are complex and are not yet fully understood. In this context, implicating the expression of p27Kip1, a cell cycle repressor protein, in these links is intriguing. On the one hand, numerous nutritional and chemopreventive anti-cancer agents specifically and consistently increase the expression of p27Kip1 thereby decreasing the risk of cancer. On the other hand, glucose (and associated energy metabolisms) and insulin (and associated receptor tyrosine kinases) specifically and consistently decrease the expression of p27Kip1 thereby increasing the risk of cancer in obesity and type 2 diabetes.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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