Role of Adiponectin in Colon Cancer Development

Hyun-Seuk Moon, PhD

Division of Endocrinology Diabetes and Metabolism,
Beth Israel Deaconess Medical Center, Harvard Medical School

Endocrinology Section, Boston VA Healthcare System,
Harvard Medical School

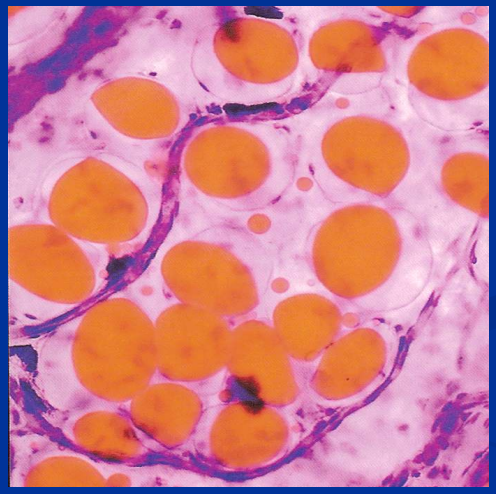
DISCLOSURE

Nothing to disclose

Outline

- **Introduction**
  - Adipocytes
  - Obesity
  - Obesity-related diseases
  - Surgical and alternative medical trails against obesity
  - Obesity-related cancers
  - Colon cancer
  - Hormone therapy for cancers
  - Adiponectin
  - LKB1 and cancers
  - AMPK and S6 signaling in cancers
  - Cell cycle regulatory and tumor suppressor genes
- **Results**
  - Part 1: Adiponectin regulates tumor weight in vivo.
  - Part 2: Adiponectin suppresses malignant potential of colon cancer cells in a LKB1 specific manner.
- **Conclusions**
- **Further Studies**
- **Acknowledgements**

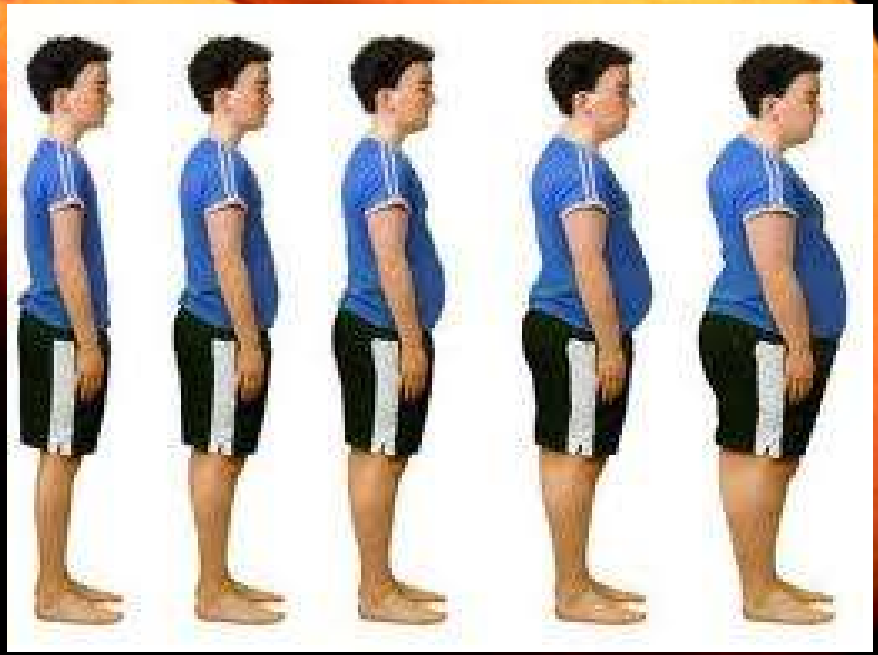
Adiopcytes



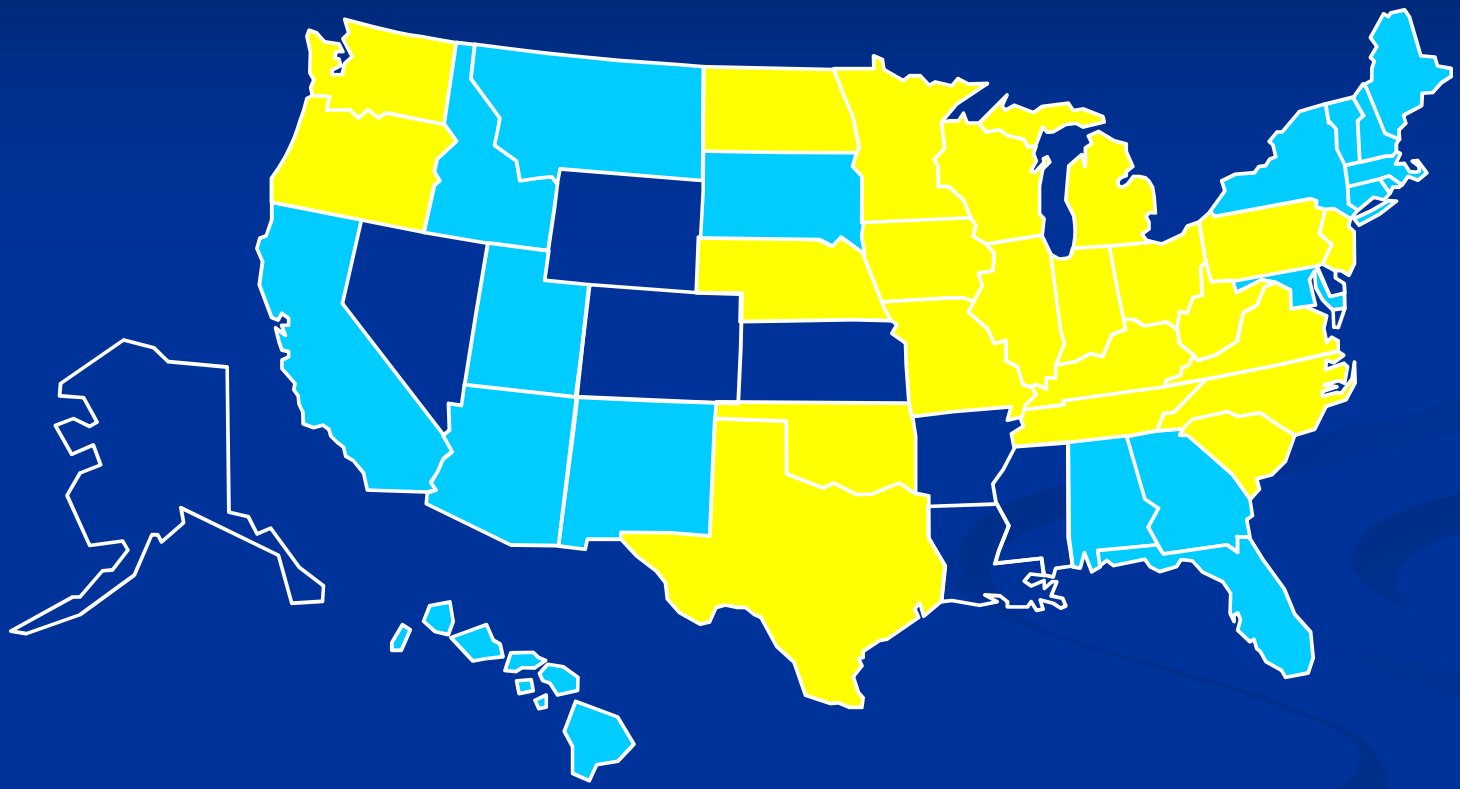
Obesity

WHY ARE WE SO

fat?



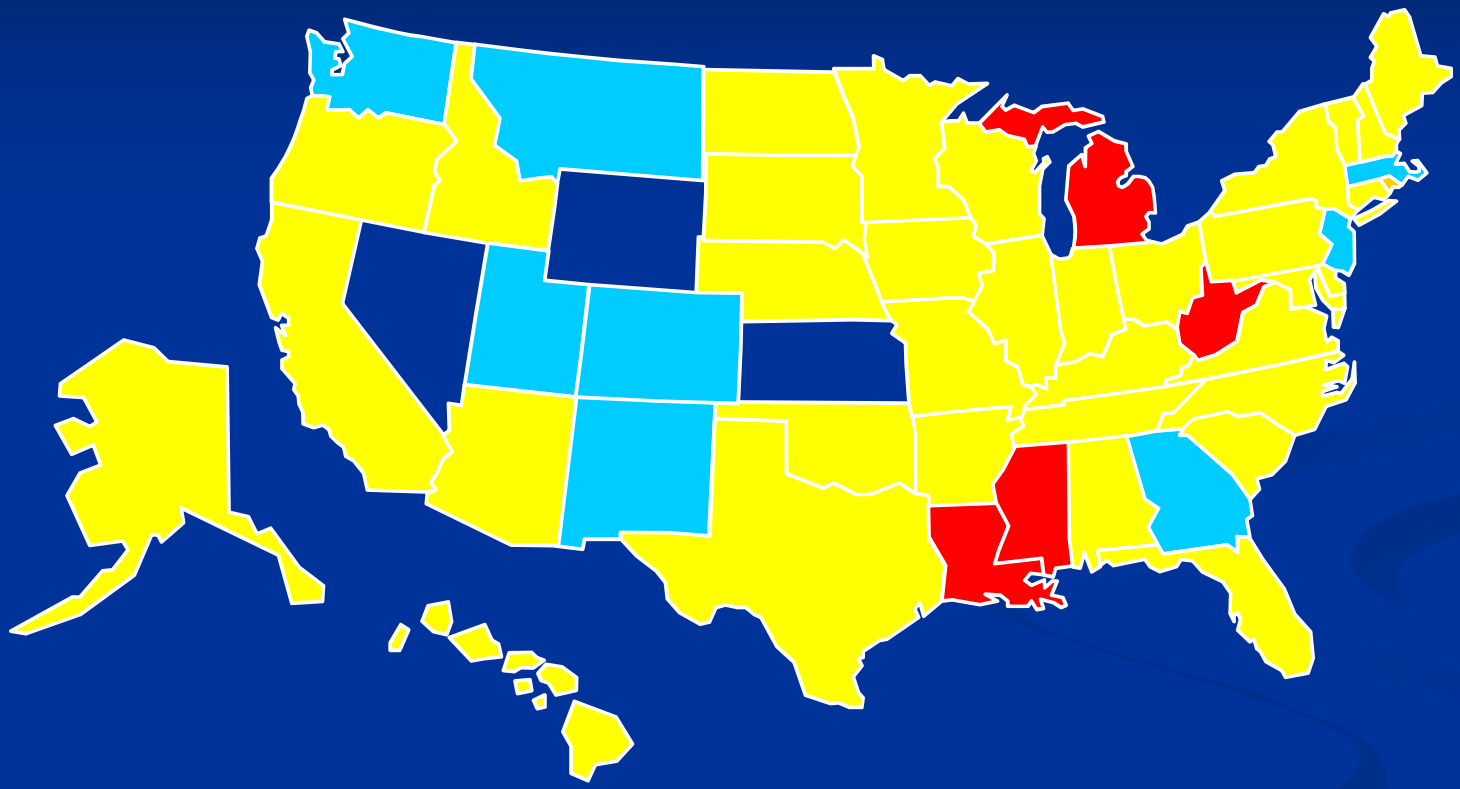
Prevalence of Obesity Among Adults:1988



< 10% 10-15% > 15%

Age-adjusted percent of adults >20 years old who are obese

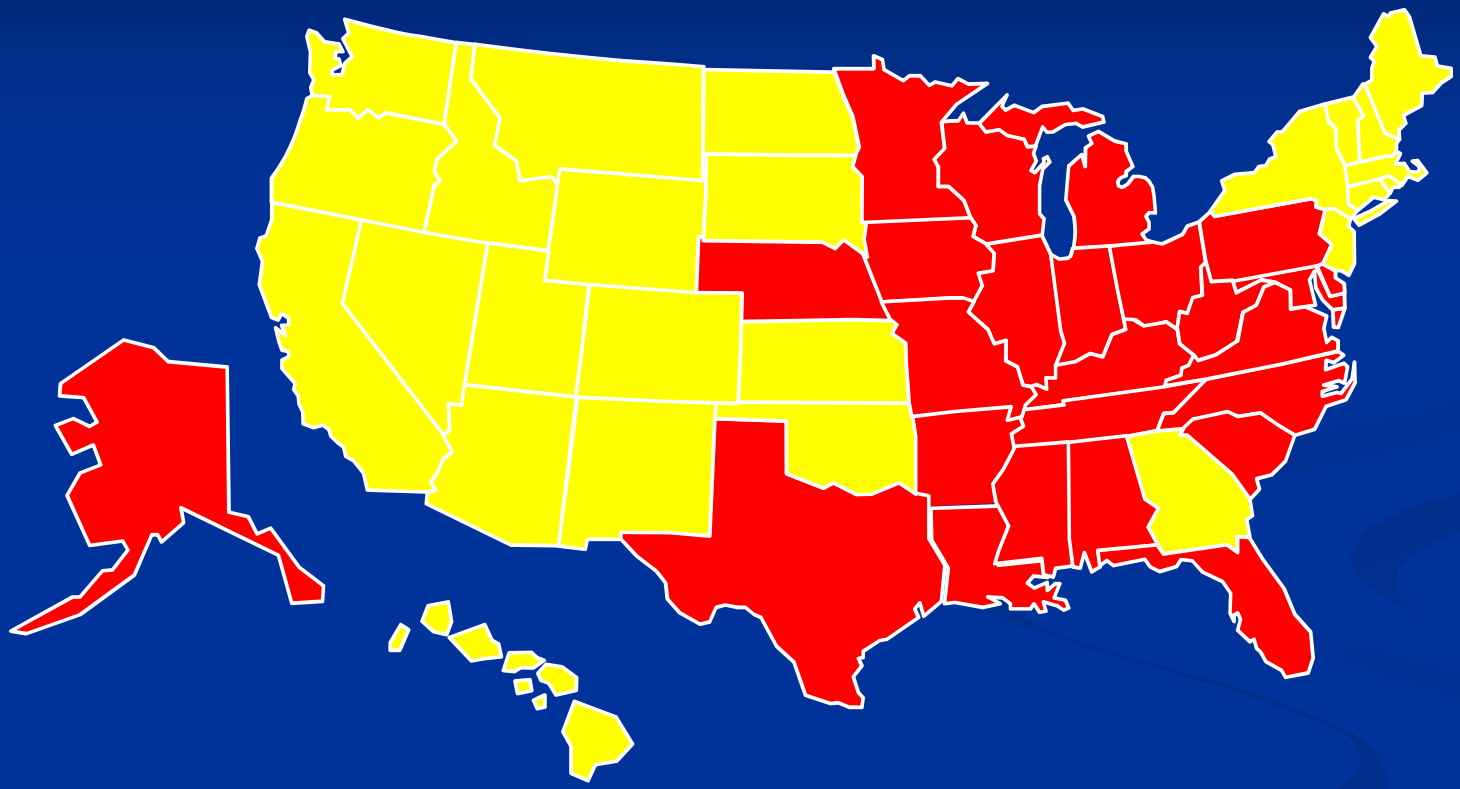
Prevalence of Obesity Among Adults:1994



< 10% 10-15% > 15%

Age-adjusted percent of adults >20 years old who are obese

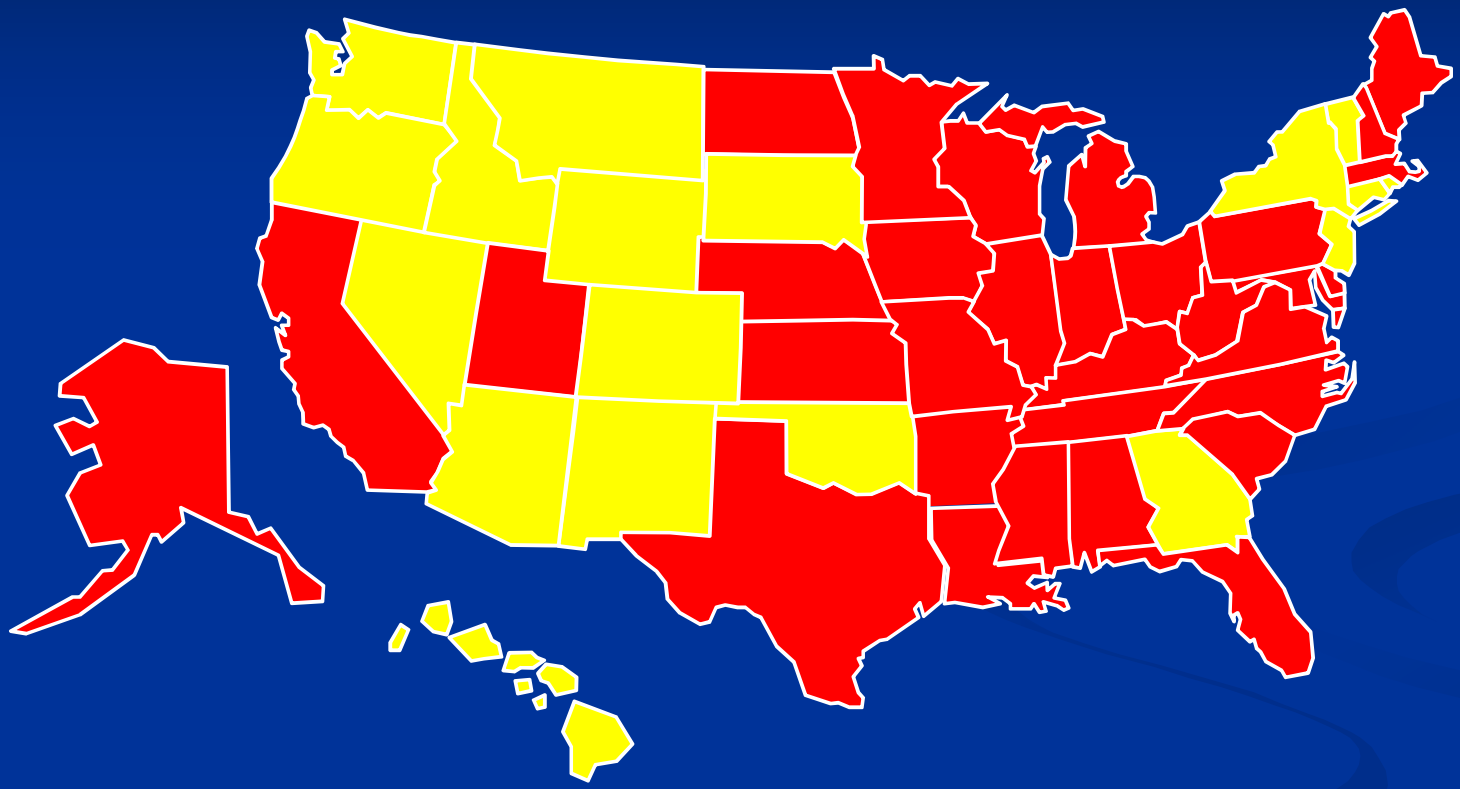
Prevalence of Obesity Among Adults:2005



< 10% 10-15% > 15%

Age-adjusted percent of adults >20 years old who are obese

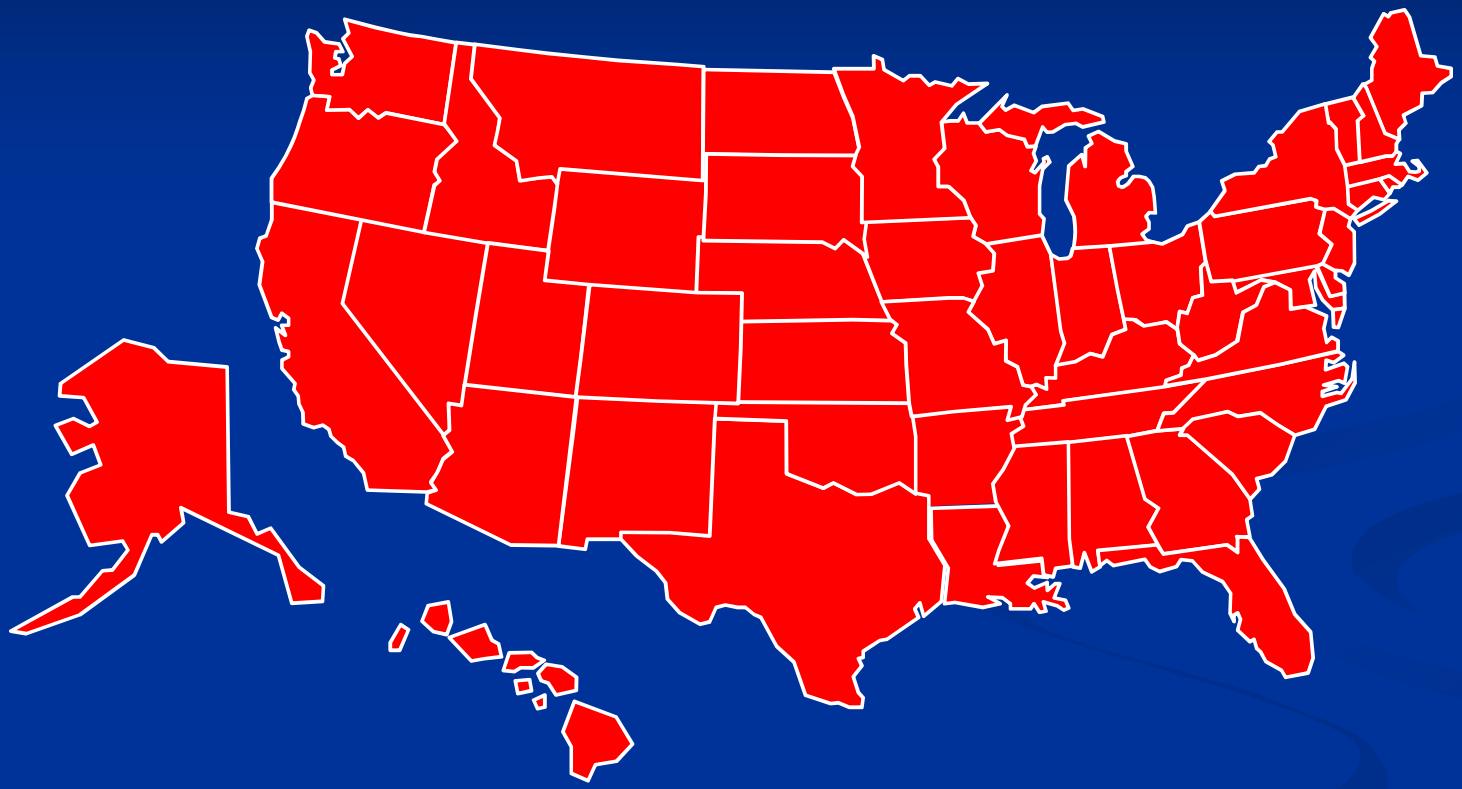
Prevalence of Obesity Among Adults:2009



< 10% 10-15% > 15%

Age-adjusted percent of adults >20 years old who are obese

2012 May Be?

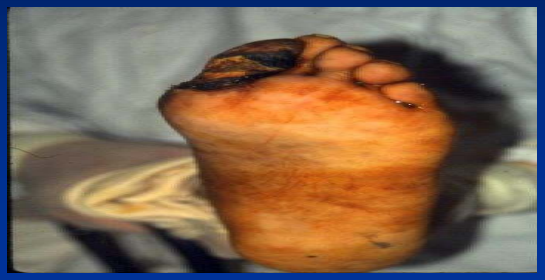


The latest National Health and Nutrition Examination Survey in the United States (2009) has found at 72.9% of the country's population is overweight

2 out 3 Adults – Over weight and/or Obese

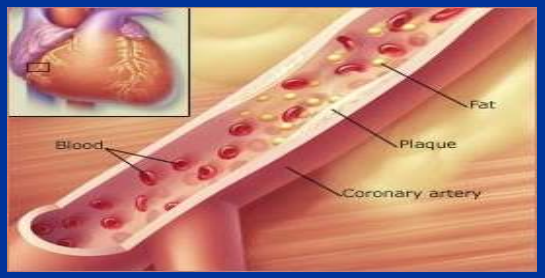
1 out of 3 Kids – Over weight and/or Obese

Obesity-related diseases



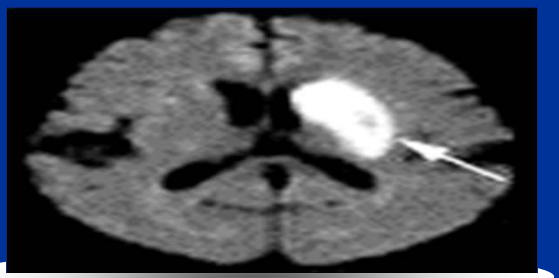
Diabetes

Hypertension



Atherosclerosis

Hyperlipidemia



Cerebral infarction

Heart attack

Surgical and alternative medical trials against obesity

Suction lipectomy

Bu-Wang

Electric stimulation

Ear chip

Heat maker

Diet tea

Lipolysis needle

Diet cookie

How many people get cancer from being overweight or obese? How many die?

- In 2007, about 41,000 new cases of cancer in the United States were estimated to be due to obesity. This means that about 3.2 percent of all new cancers are linked to obesity.

- A recent report estimated that, in the United States, 14 percent of deaths from cancer in men and 20 percent of deaths in women were due to overweight and obesity.

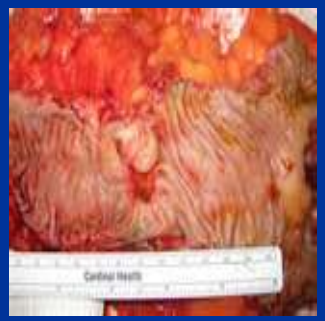
from National Cancer Institute

Developing Cancer from Obesity

- Endometrial cancer
- Breast cancer
- Kidney cancer
- Liver cancer
- Ovarian cancer
- Pancreas cancer
- Uterine cancer
- **Colon cancer**

from American Cancer Society

Colon cancer



- Colon cancer is one of the most prevalent malignancies, ranking as the second leading cause of death from cancer in the United States.

- Colon cancer occurs more frequently in people who are obese than in those of a healthy weight.

- An increased risk of colon cancer has been reported for men with high BMIs.

from National Cancer Institute

Adiponectin

- Adiponectin, adipocyte-secreted hormone, modulates a number of metabolic processes.
  - Berg et al. Nat Med, 2001
- Reduced levels of adiponectin are associated with cancer risk.
  - Dal Maso et al. J Clin Endocrinol Metab 2004
- Adiponectin has anti-proliferative and pro-apoptotic effects on breast cancer cells.
  - Kang et al. Arch Pharm Res, 2005
- Adiponectin suppresses prostate cancer development via AMPK activation and subsequent inhibition of mTOR.
  - Barb et al. Endocrine-related cancer, 2007
- Adiponectin inhibits cell growth and induces apoptosis in endometrial cancer.
  - Moon et al. Molecular Cancer Theraceutics 2011

Adiponectin might be useful as a potential therapeutic agent for cancers.

The purpose of the study

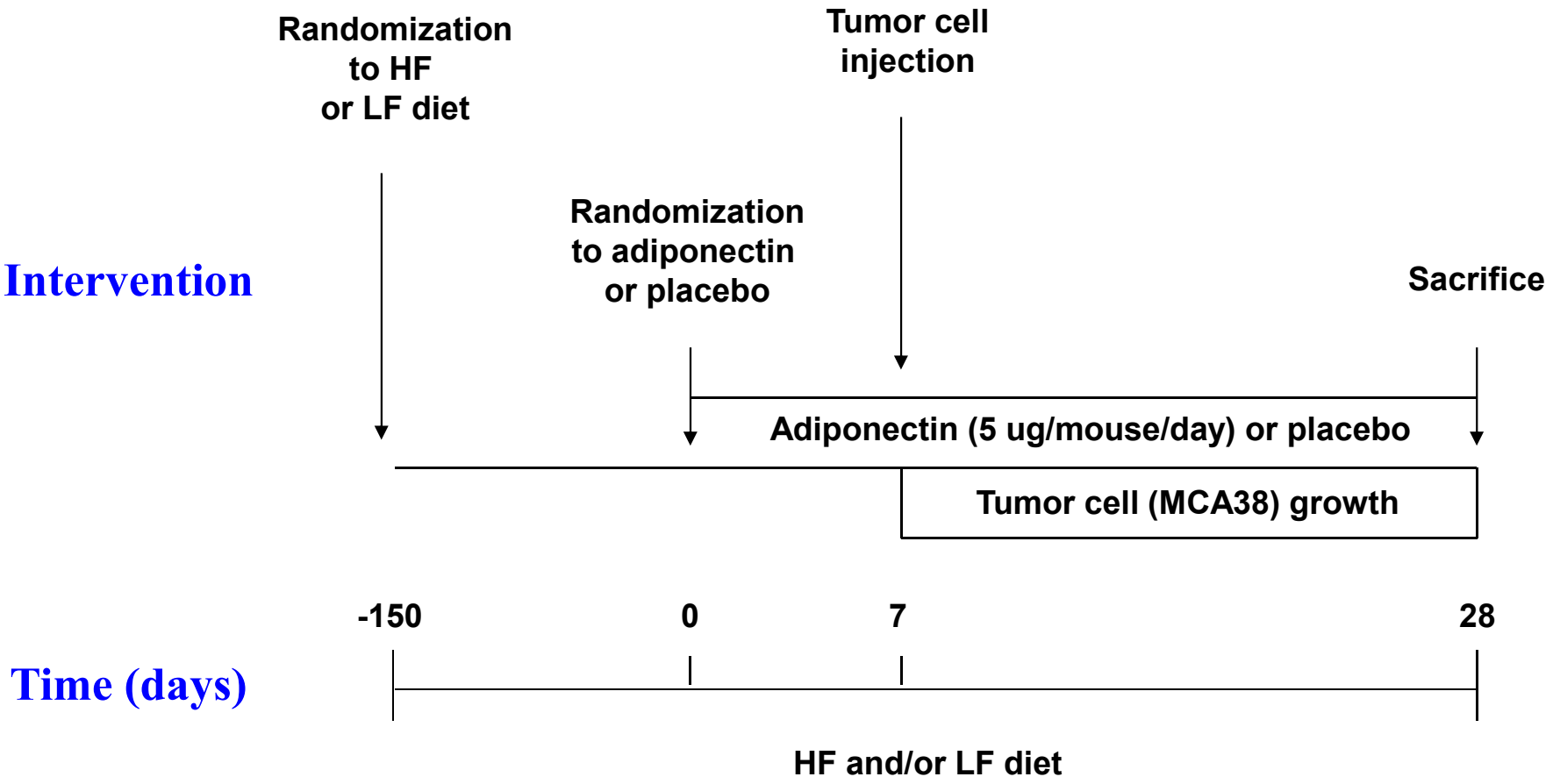
- A mechanistic/chemoprevention study of adiponectin in APN WT and APN-in mice in conjunction with western diet or regular diet has not been reported.

- No previous study has investigated the underlying mechanism *in vivo* or tested in details *in vitro* in colon cancer.

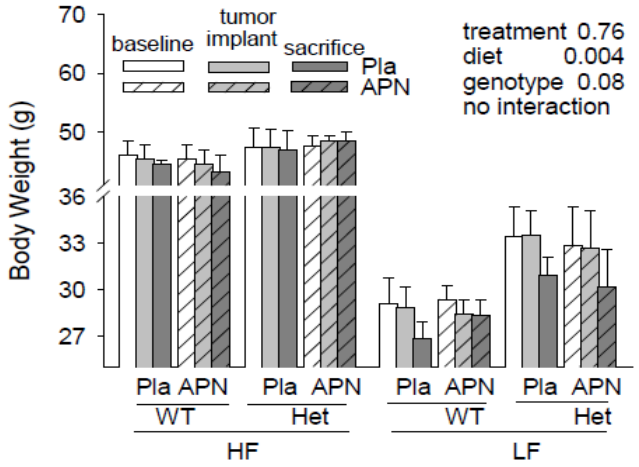
To address these questions, we designed a translational study to investigate the potential anti-cancer effect of adiponectin on colon cancer *in vivo* and *in vitro*.

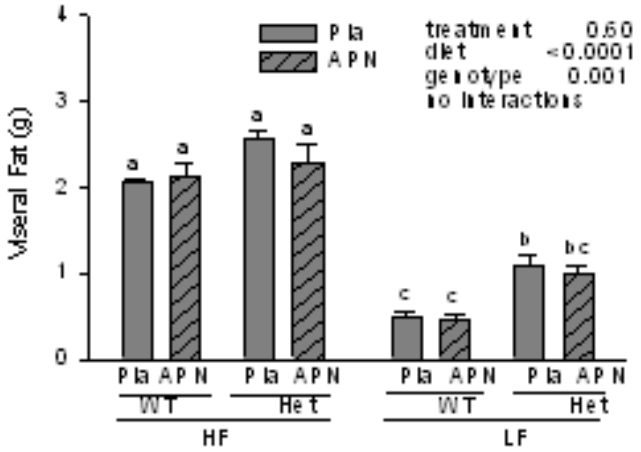
Part 1: *In vivo* Animal Study

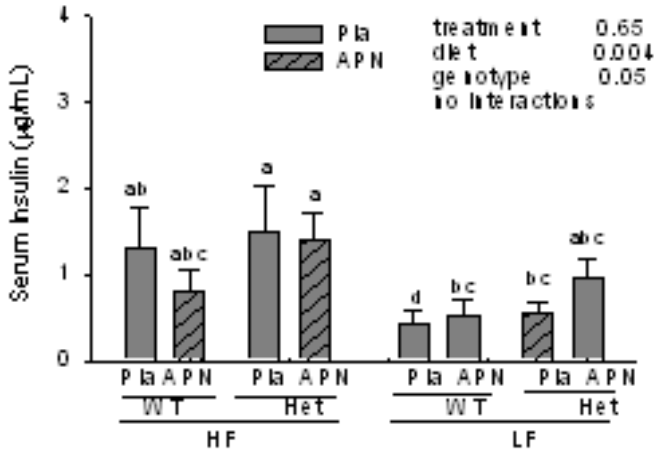
Experimental Design

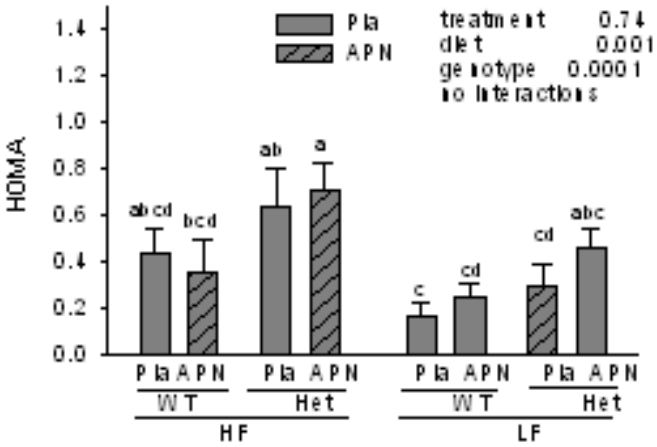


Adiponectin treatment has no effect on body weight and visceral fat

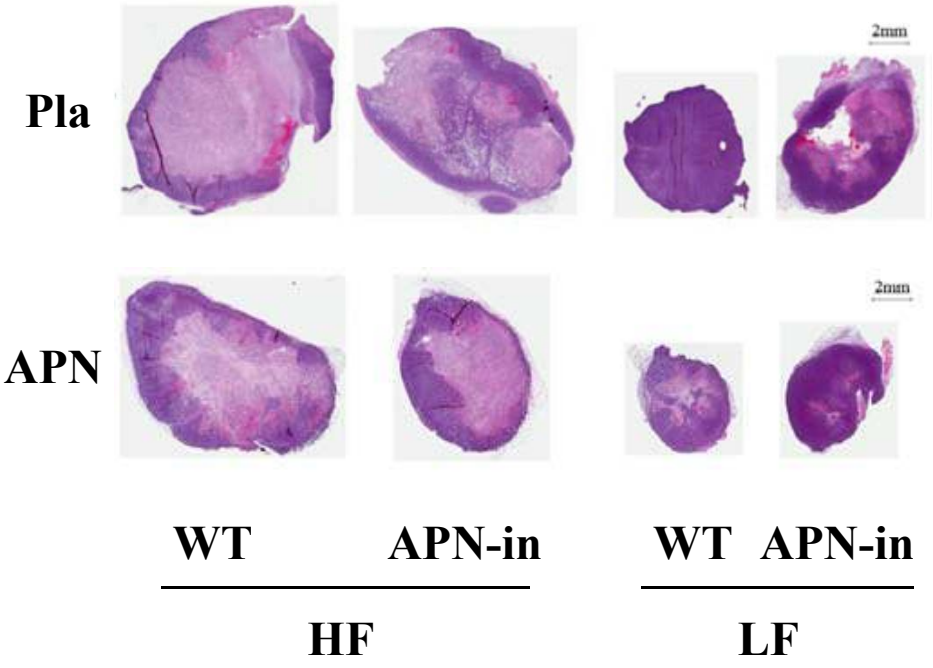


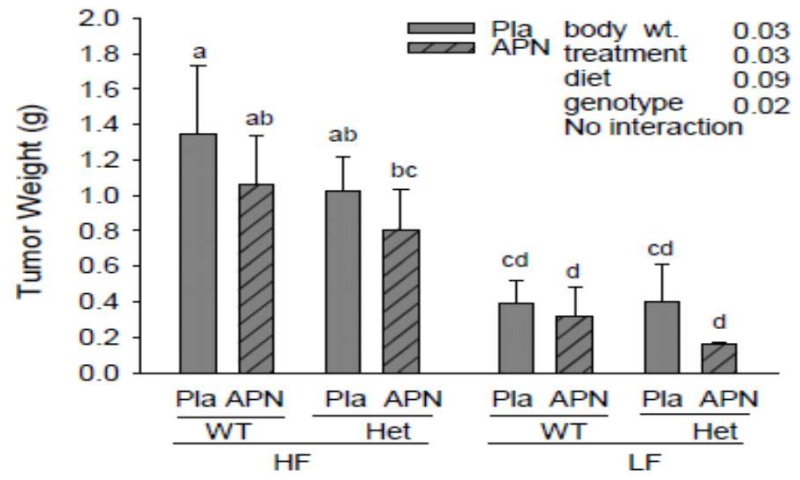


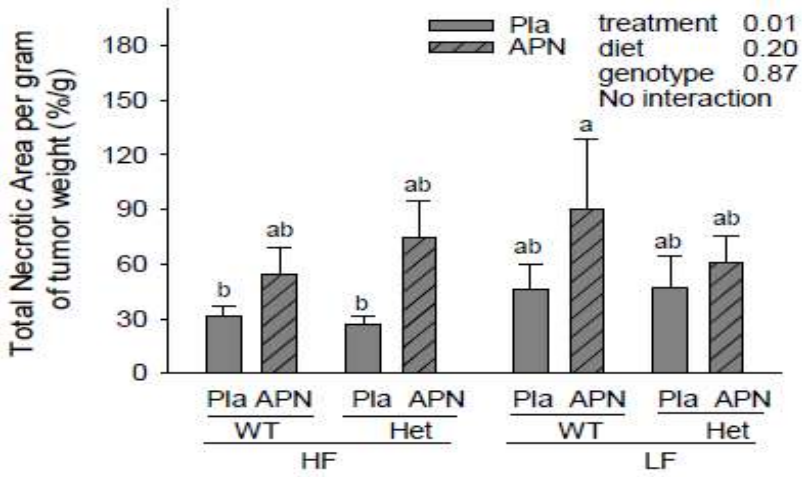




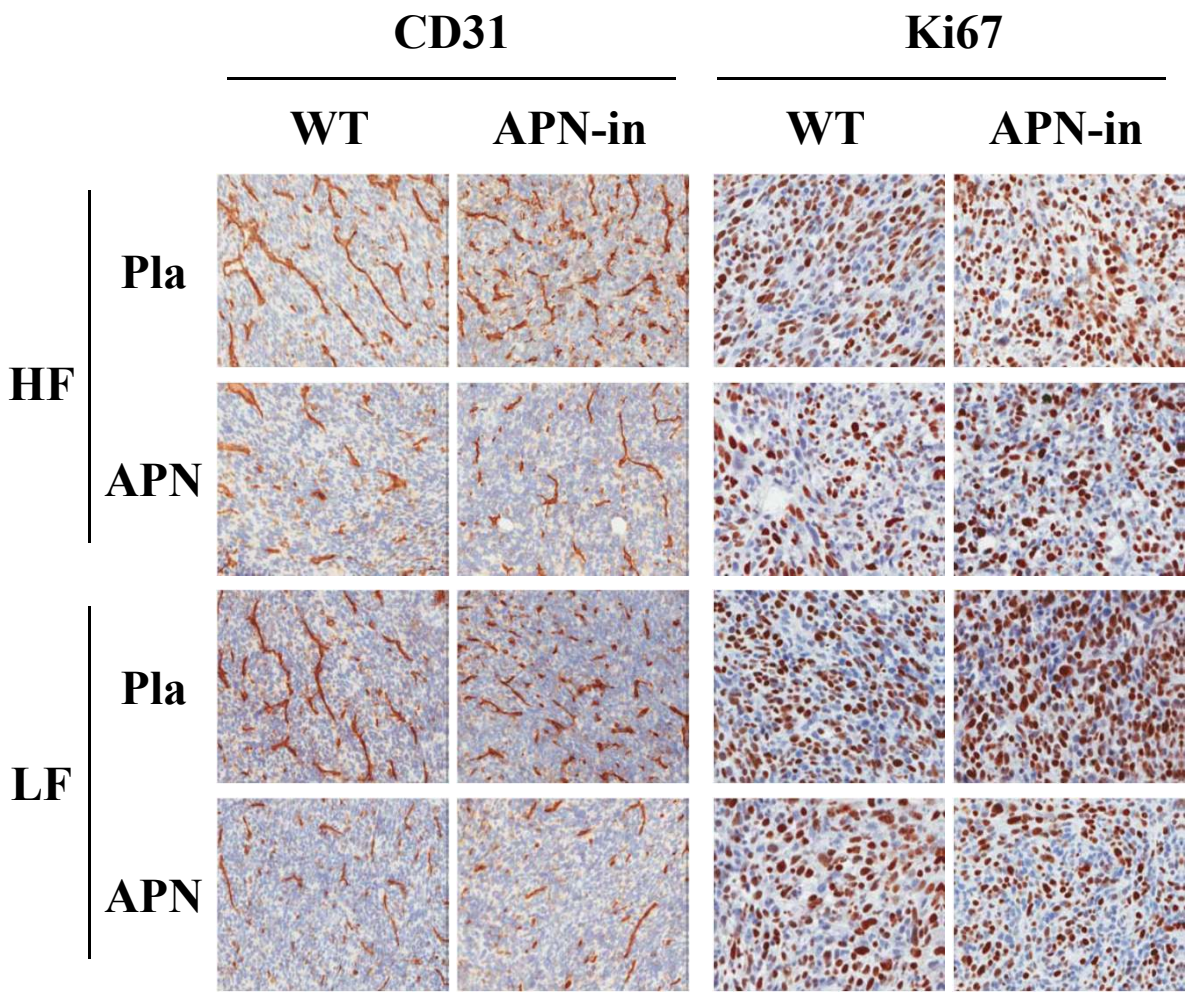
Adiponectin treatment inhibits colon cancer growth and caused extensive central necrosis in WT and APN-in mice fed on either HF or LF diet

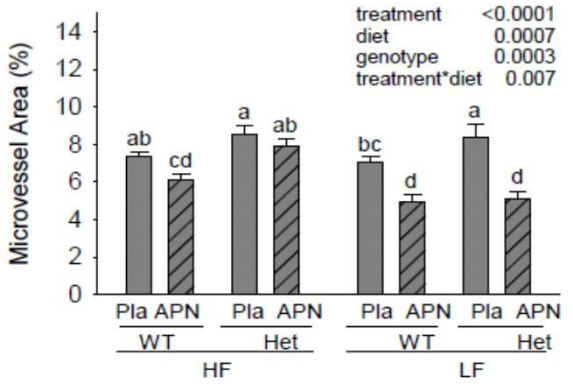


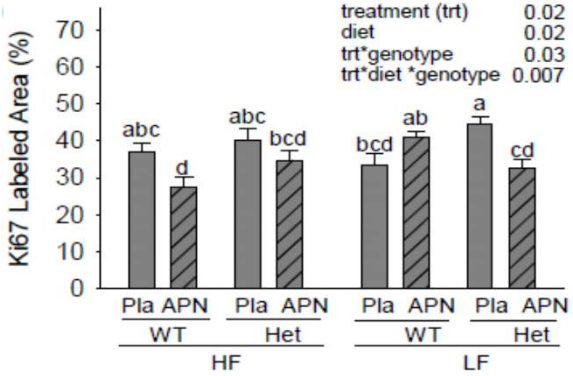




Adiponectin treatments inhibits tumor growth by reducing the expression of angiogenic and proliferation markers in WT and APN-in mice fed on either HF or LF diet







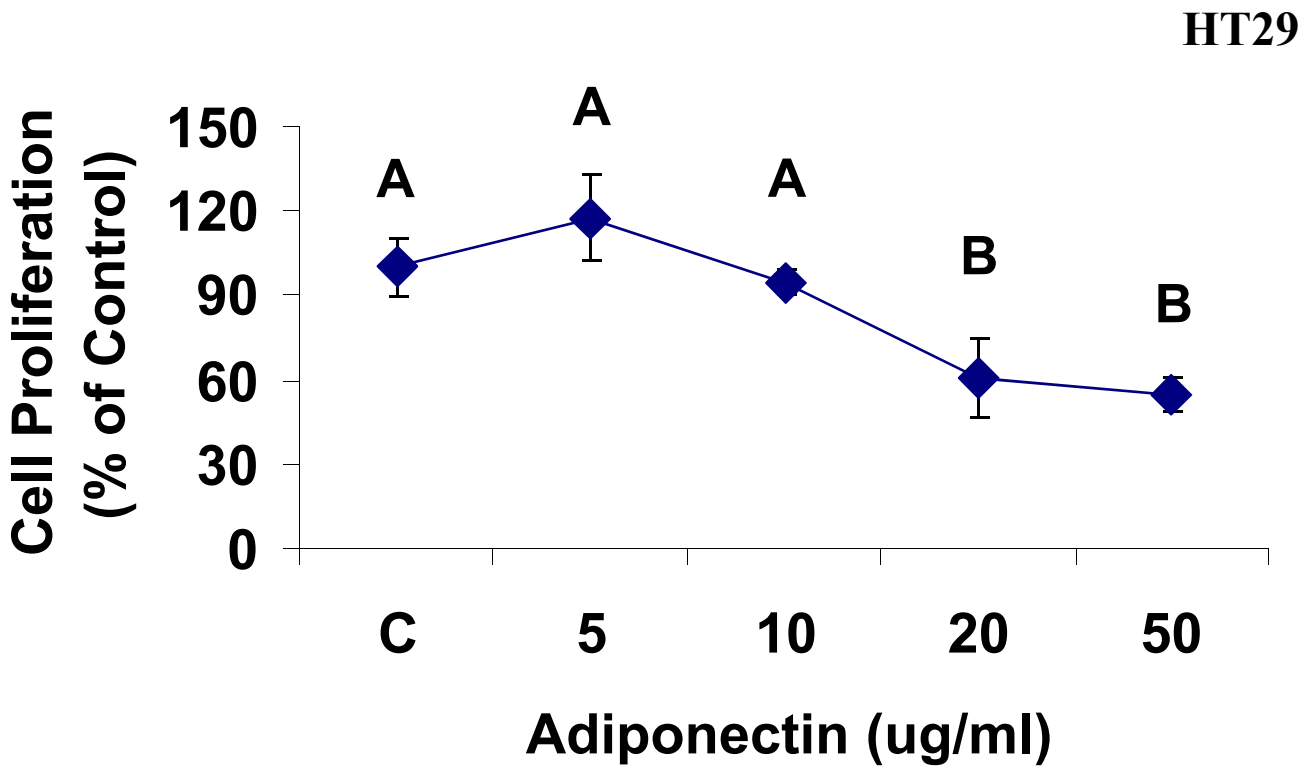
Summary 1

- HF diet mice have higher body weight when compared to LF diet mice.
- Although the body weight differences between WT and APN-in mice became non significant, APN-in mice have more visceral fat when compared to WT mice.
- Adiponectin-treated mice more decreased insulin levels compared to placebo-treated mice in HF diet mice.
- HF diet mice has larger tumors when compared to LF diet mice.
- Adiponectin increased central necrotic areas and decreased tumor sizes compared to placebo-treated mice.
- Adiponectin-treated mice have less population of microvessel areas when compared to placebo-treated mice.
- Adiponectin-treated mice have less Ki67 staining cells when compared to placebo-treated mice.

Part 2: *In vitro* Study

HT29 human colon cancer cell lines

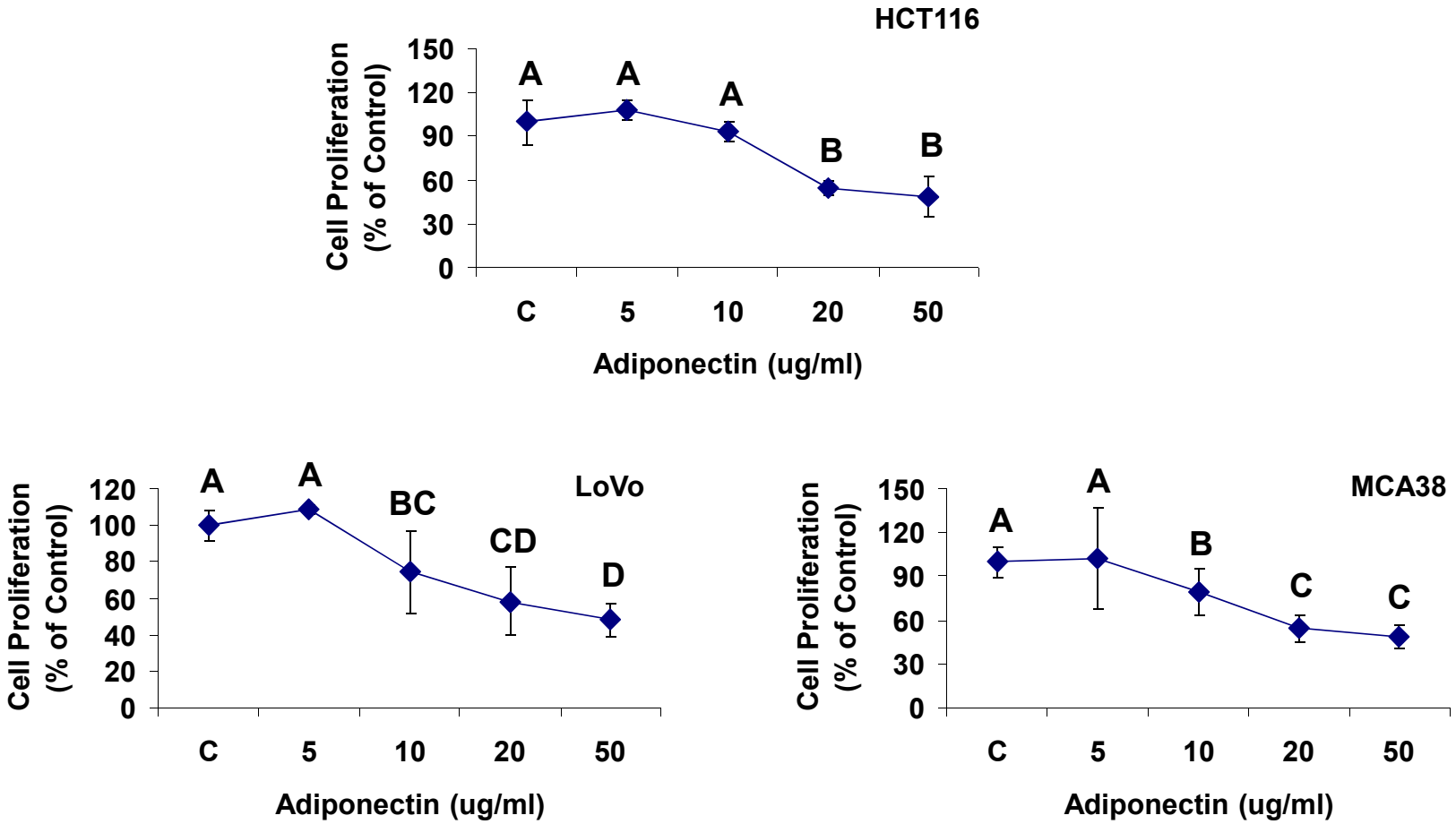
Cell Proliferation Assay



Cells were treated with adiponectin at indicated concentrations for 24hr.
Cell viability was measured using the MTT proliferation kit according to the manufacture's protocol.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.

HCT116, LoVo and MCA38 colon cancer cell lines

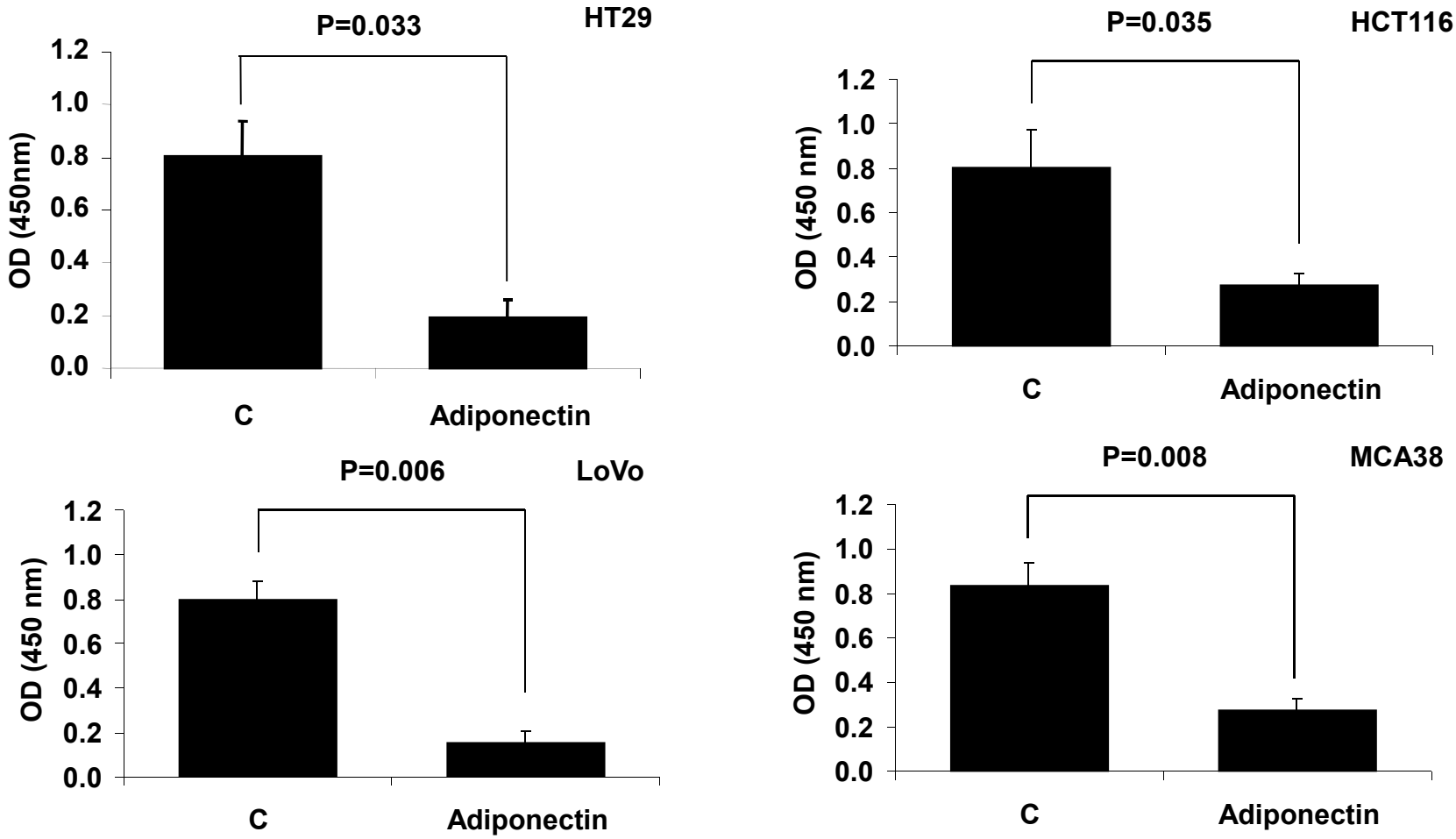
Cell Proliferation Assay



Cells were treated with adiponectin at indicated concentrations for 24hr.
Cell viability was measured using the MTT proliferation kit according to the manufacture's protocol.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.

Human and Mouse colon cancer cell lines

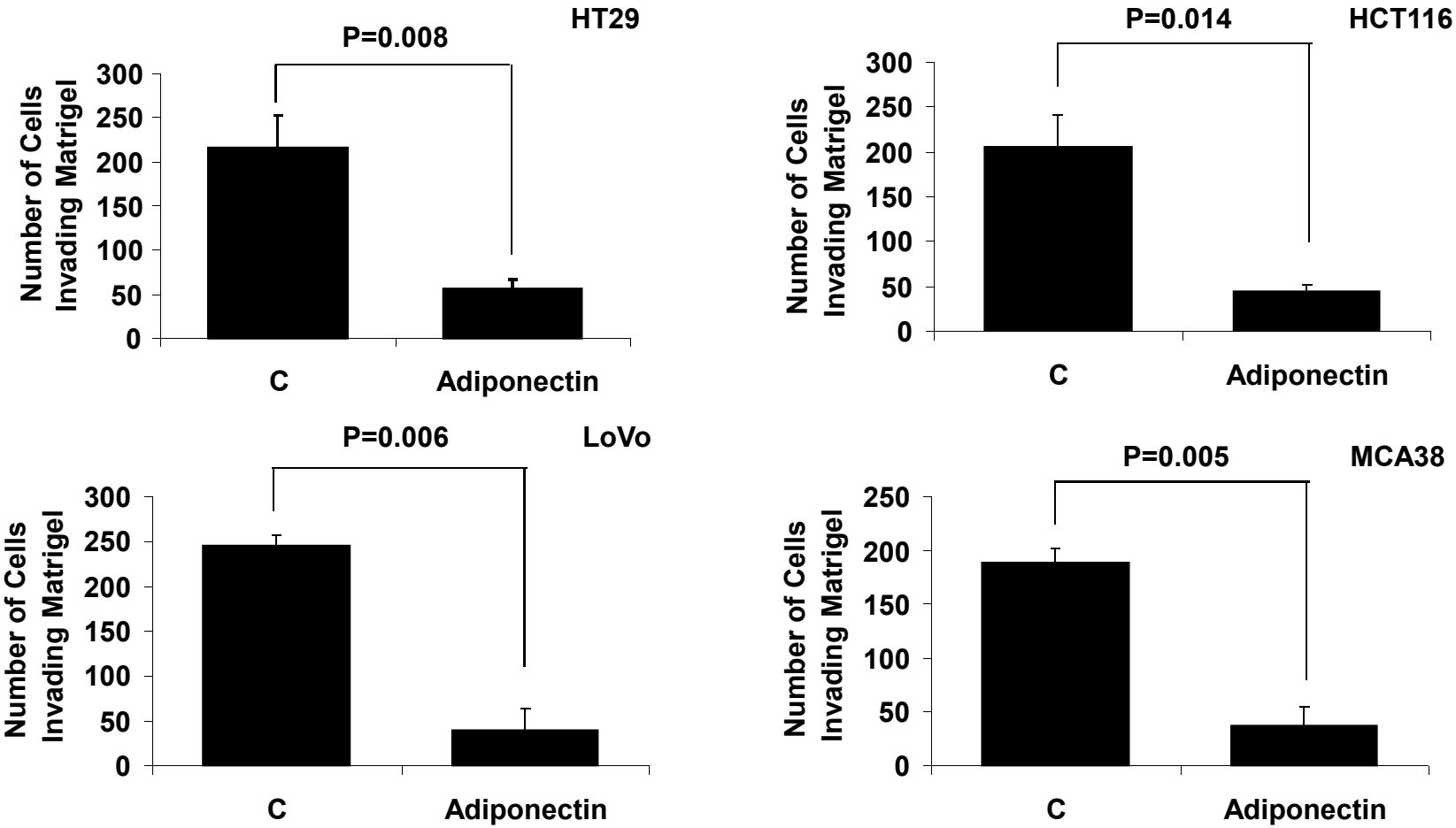
Cell Adhesion Assay



Cells were pretreated with 20µg/ml of adiponectin for 24hr and plated (5 × 10^4 cells) in 10µg/cm^2 fibronectin-coated wells in 96-well plates followed by 60min incubation at 37°C (5% CO^2). Adherent cells were fixed with 3% paraformaldehyde for 10min, washed with 2% methanol for 10min and stained with 0.5% crystal violet in 20% methanol for 10min. The stain was eluted and absorbance at 540 nm was measured. Data were analyzed using Student *t*-test. Values are means (n=3) ± SD

Human and Mouse colon cancer cell lines

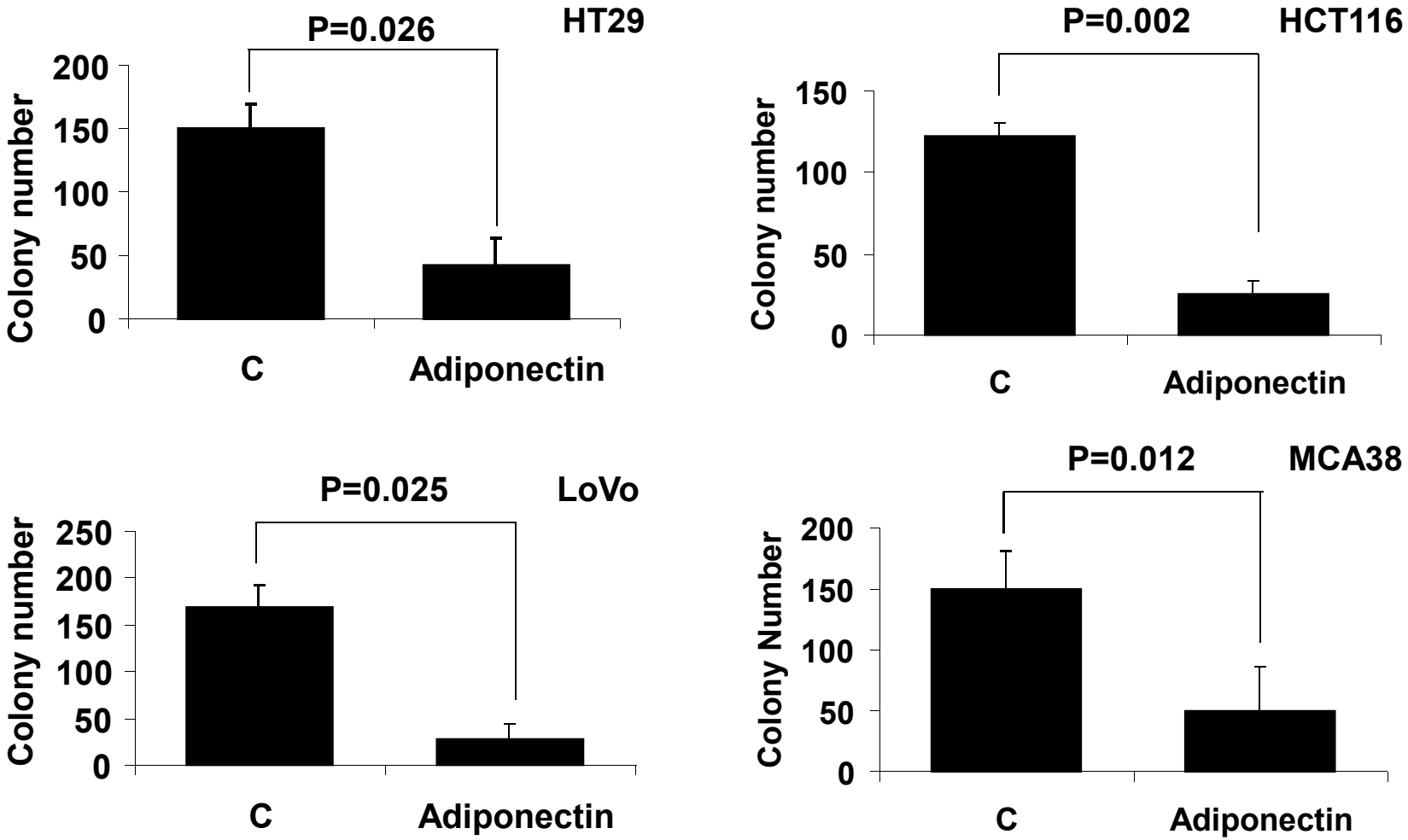
Matrigel Cell Invasion Assay



For an *in vitro* model system for metastasis, Matrigel invasion assay by using a Matrigel invasion chamber from BD BioCoat Cellware was performed.
Data were analyzed using Student *t*-test. Values are means (n=3) ± SD

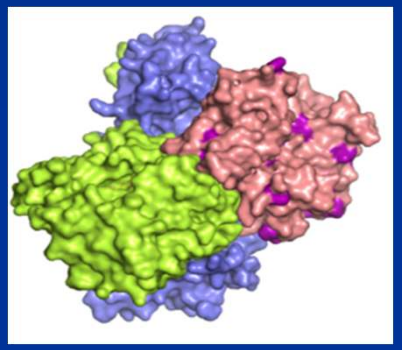
Human and Mouse colon cancer cell lines

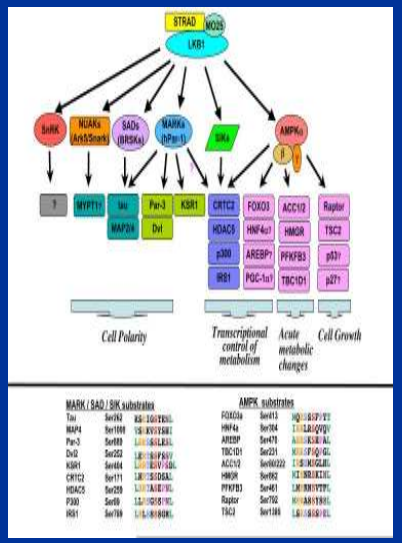
Clonogenic Assay



The cells were seeded in 10 cm plates at a density of 500 cells per well overnight. The following day, cells were treated with 20µg/ml of adiponectin and the medium was replaced with fresh medium containing adiponectin every 3 days. After a 10-day treatment period, the medium was removed and cell colonies were stained with crystal violet (0.1% in 20% methanol). Colonies containing >50 normal-appearing cells were counted.
Data were analyzed using Student *t*-test. Values are means (n=3) ± SD

LKB1



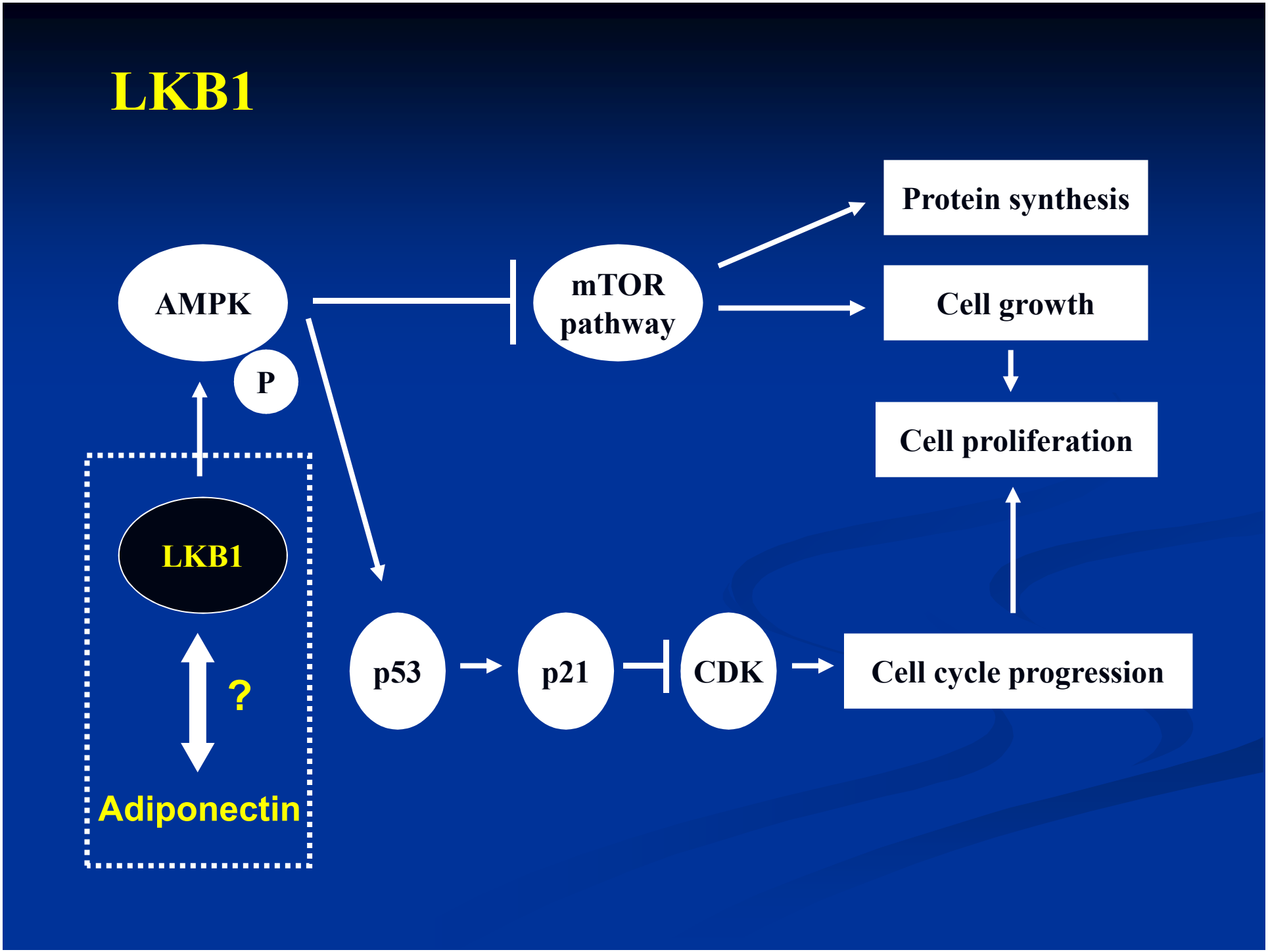


- *LKB1* is a necessary element in cell metabolism that is required for maintaining energy homeostasis.

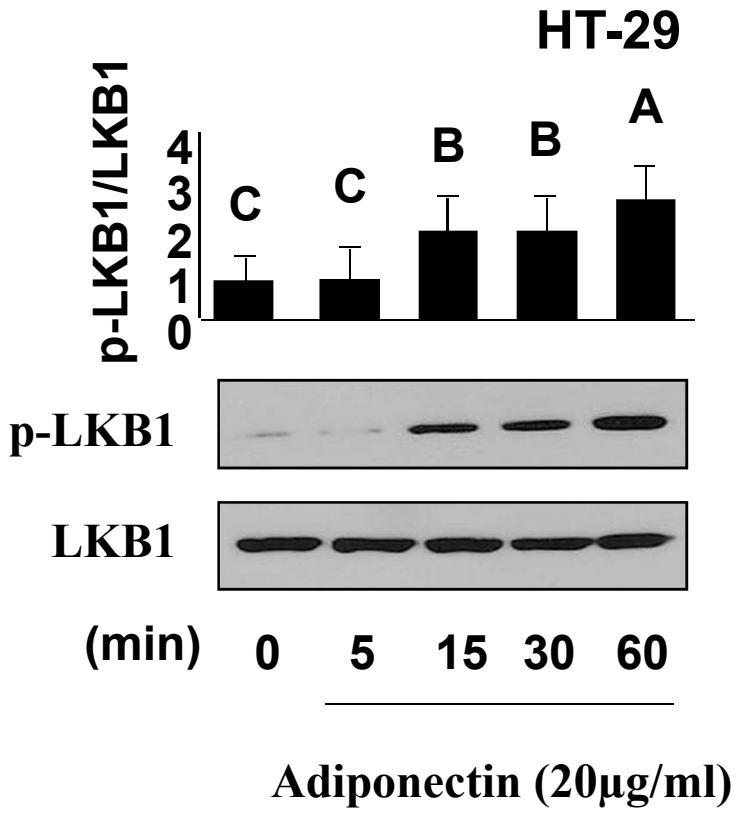
  - Shae et al. 2004 Cancer Cell

- *LKB1* regulates cell polarity and functions as tumor suppressor.

  - Baas et al. 2003 EMBO J

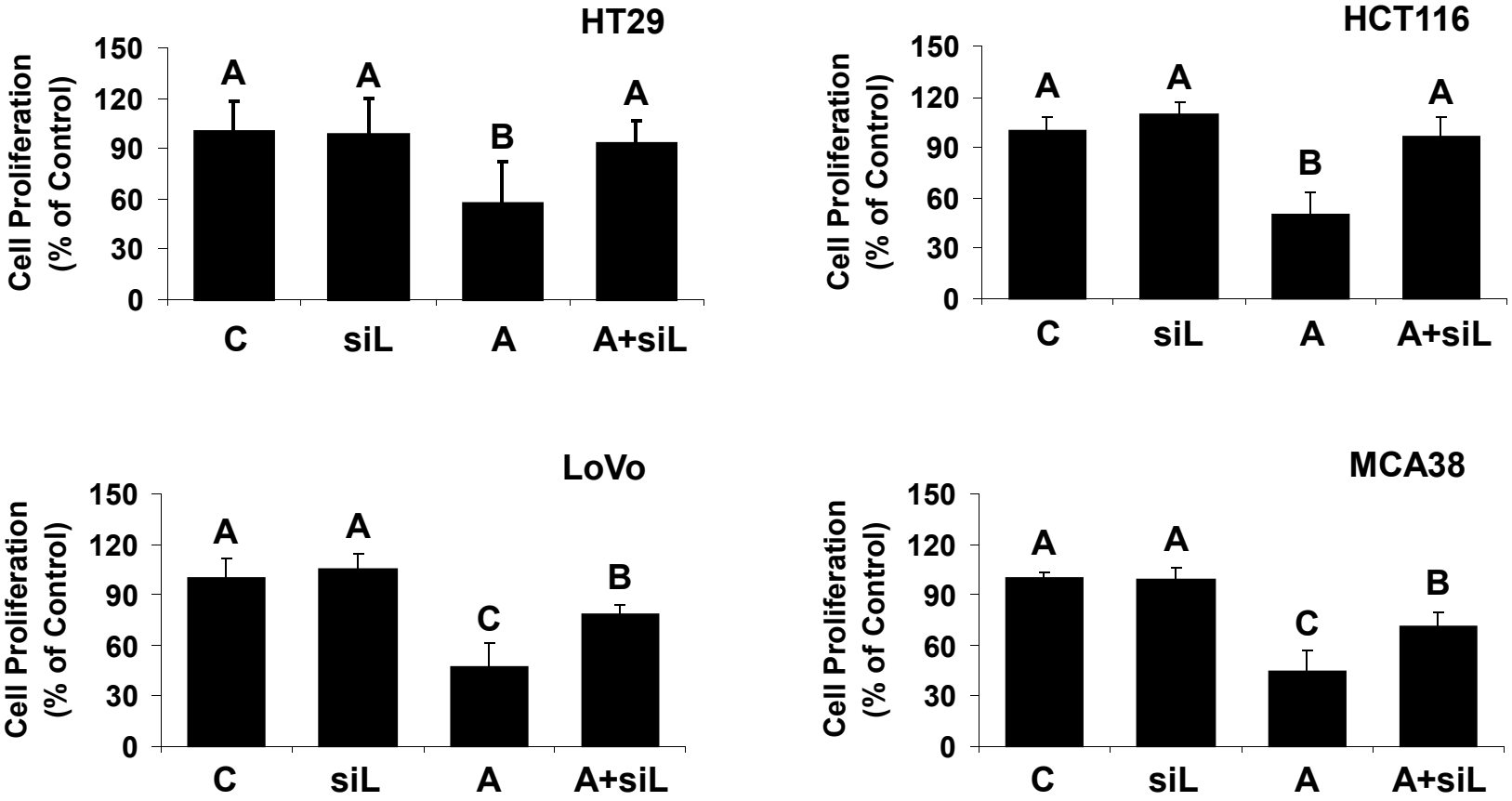

LKB1
Protein synthesis
AMPK
mTOR pathway
Cell growth
P
Cell proliferation
LKB1
p53
p21
CDK
Cell cycle progression
?
Adiponectin

Adiponectin mediates activation of LKB1 in human colon cancer cells



Human and Mouse colon cancer cell

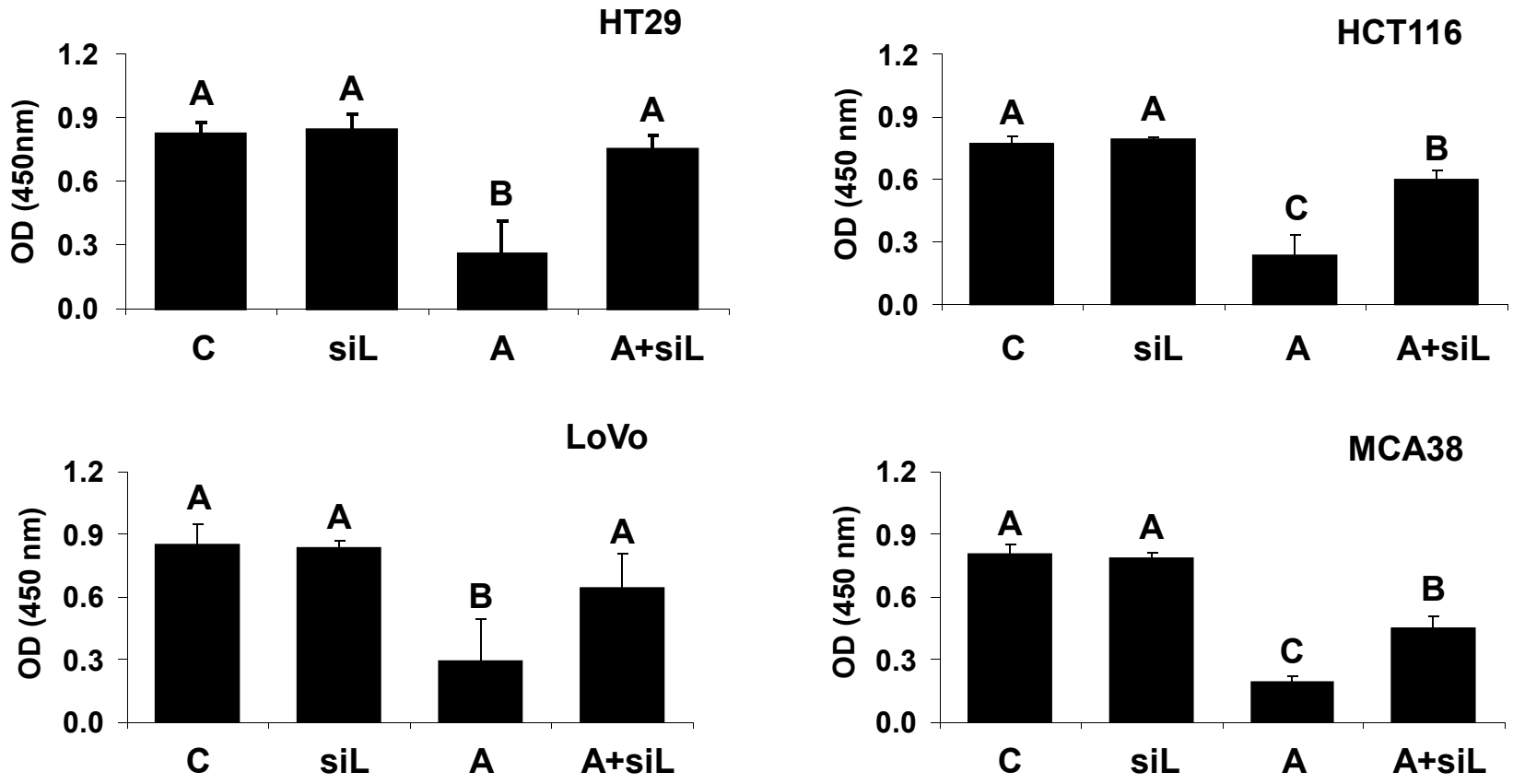
Cell Proliferation Assay with LKB1 siRNA administration



Cells were transfected with LKB1 siRNA for 5hr and then treated with adiponectin (20ug/ml) for 24hr.
Cell viability was measured using the MTS proliferation kit according to the manufacture's protocol.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.
C: Control, A: Adiponectin, siL: LKB1 siRNA

Human and Mouse colon cancer cell lines

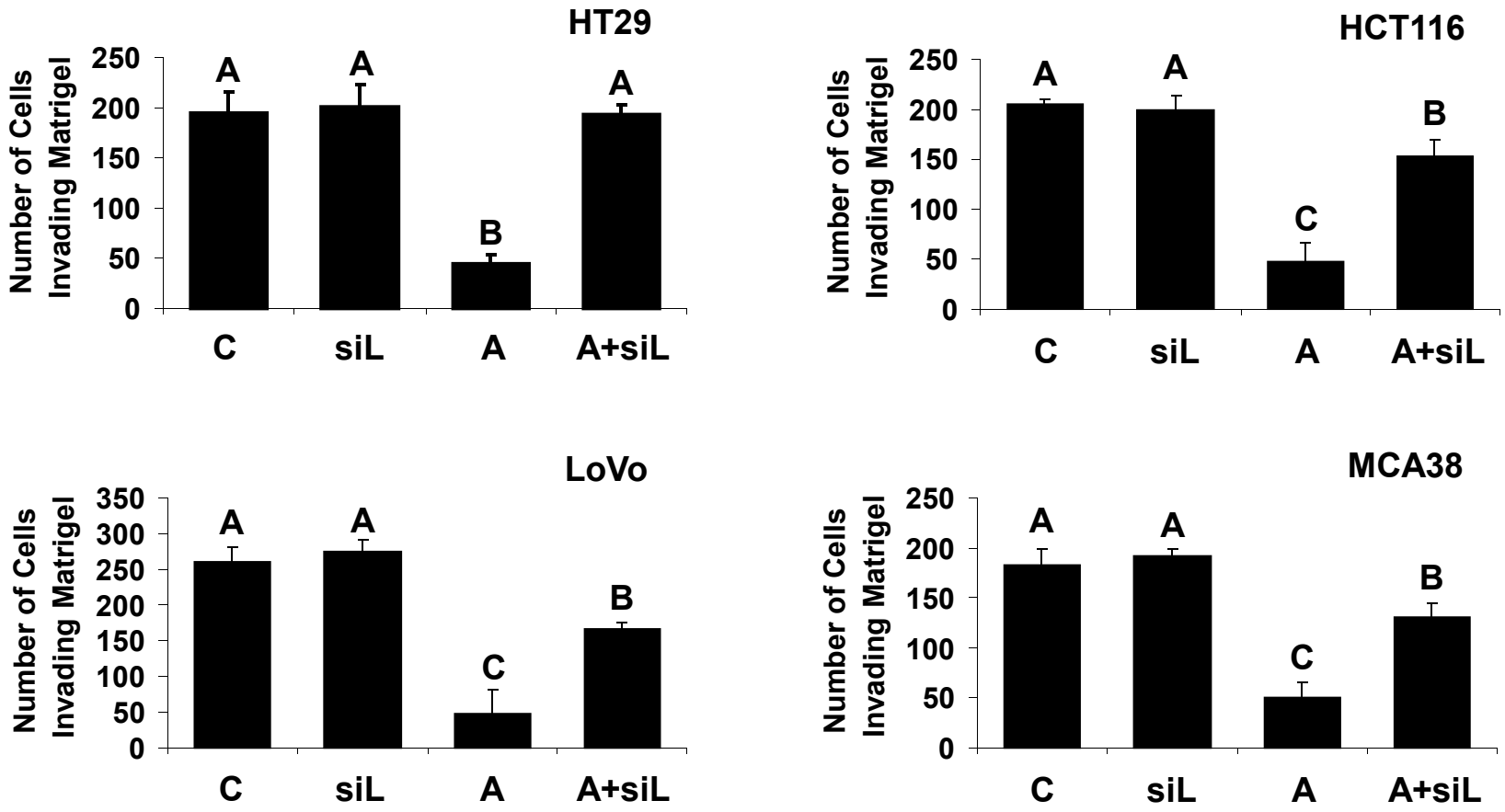
Cell Adhesion Assay with LKB1 siRNA administration



Cells were trnasfected with LKB1 siRNA for 5hr, treated with 20μg/ml of adiponectin for 24hr, and plated (5 × 10^4 cells) in 10μg/cm^2 fibronectin-coated wells in 96-well plates followed by 60min incubation at 37°C (5% CO^2). Adherent cells were fixed with 3% paraformaldehyde for 10min, washed with 2% methanol for 10min and stained with 0.5% crystal violet in 20% methanol for 10min. The stain was eluted and absorbance at 540 nm was measured.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.
C: Control, A: Adiponectin, siL: LKB1 siRNA

Human and Mouse colon cancer cell lines

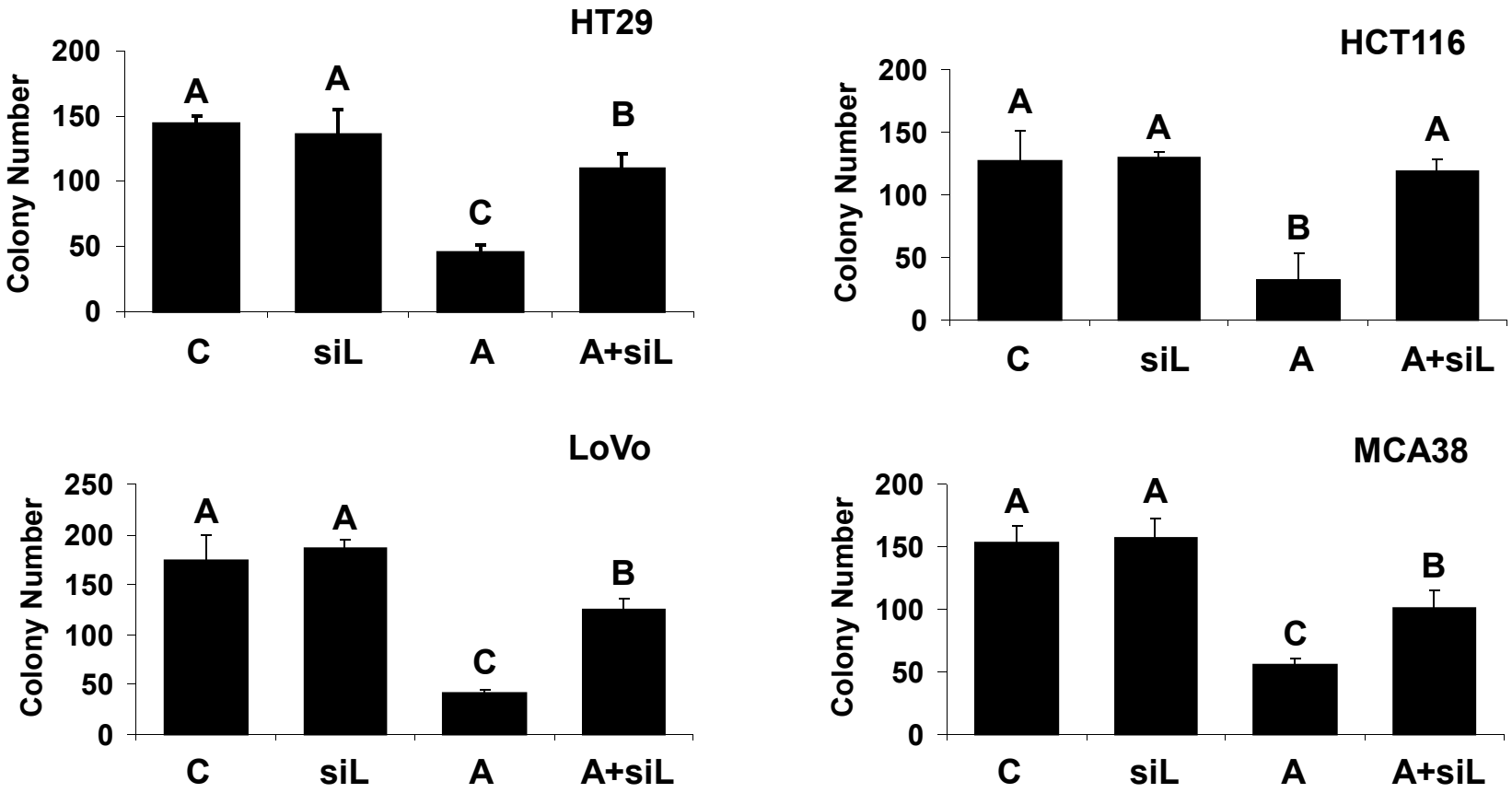
Matrigel Cell Invasion Assay with LKB1 siRNA administration



For an *in vitro* model system for metastasis, Matrigel invasion assay by using a Matrigel invasion chamber from BD BioCoat Cellware was performed.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.
C: Control, A: Adiponectin, siL: LKB1 siRNA

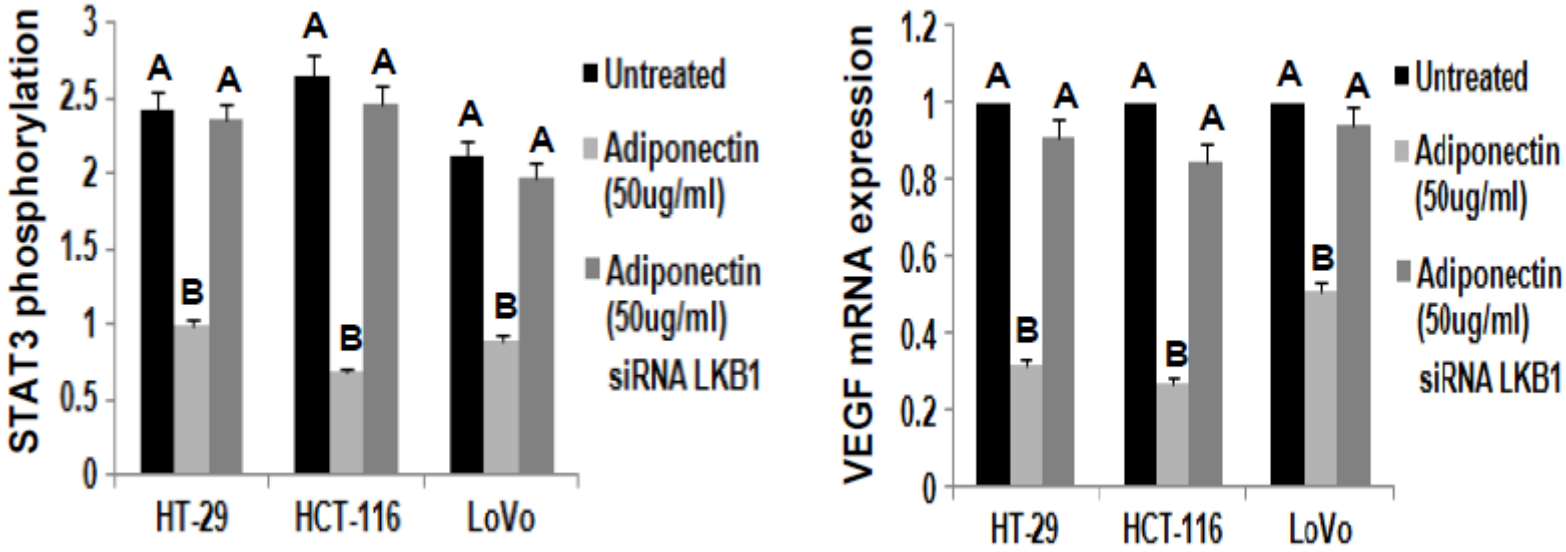
Human and Mouse colon cancer cell lines

Cell Clonogenic Assay with LKB1 siRNA administration



The cells were seeded in 10 cm plates at a density of 500 cells per well overnight. The following day, cells were transfected with LKB1 siRNA for 5hr and then treated with 20μg/ml of adiponectin and the medium was replaced with fresh medium containing adiponectin every 3 days. After a 10-day treatment period, the medium was removed and cell colonies were stained with crystal violet (0.1% in 20% methanol). Colonies containing >50 normal-appearing cells were counted.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.
C: Control, A: Adiponectin, siL: LKB1siRNA

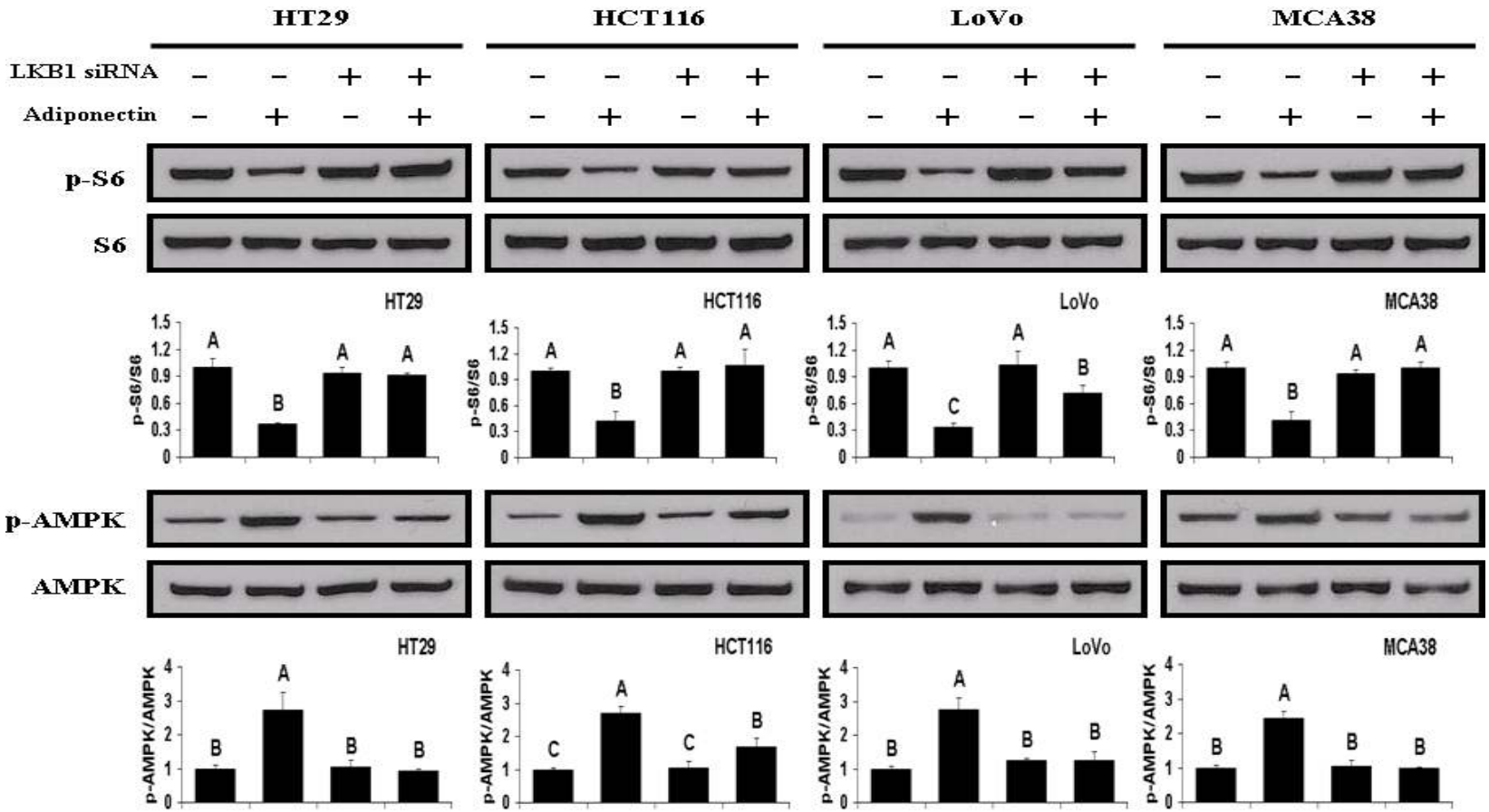
HT29, HCT116, LoVo and MCA38 colon cancer cell lines with LKB1 siRNA administration



A number of immunosuppressive factors produced by tumor cells in a STAT3-dependent manner are angiogenic factors, including VEGF.

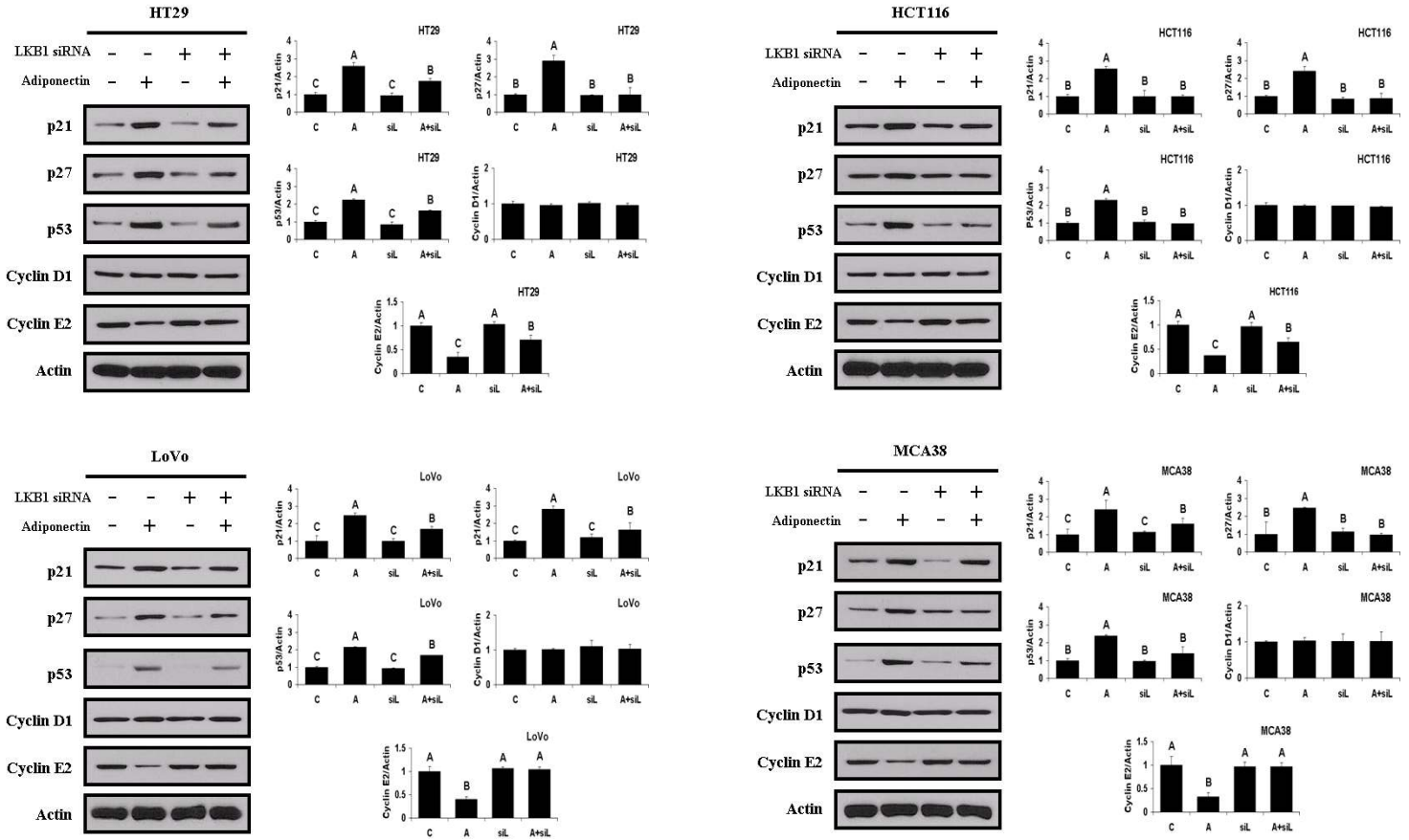
The cell were trnasfected with LKB1 siRNA for 5hr and then treated with adiponectin (50ug/ml) for 30min and/or 24 hr.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.

HT29, HCT116, LoVo and MCA38 colon cancer cell lines with LKB1 siRNA administration



The cell were trnasfected with LKB1 siRNA for 5hr and then treated with adiponectin (20ug/ml) for 30min.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.
AMPK activation can induce apoptosis in cancer cells.
S6 activation leads to an increase in protein synthesis and cell proliferation in cancer cells.

HT29, HCT116, LoVo and MCA38 colon cancer cell lines with LKB1 siRNA administration



The cell were trnasfected with LKB1 siRNA for 5hr and then treated with adiponectin (20ug/ml) for 24hr.
All data were analyzed using one-way ANOVA followed by post-hoc test for multiple comparisons.
Values are means (n=3) ± SD. Means with different letters are significantly different, $p<0.05$.

Summary 2

- Adiponectin decreased cell proliferation of human and mouse colon cancer cell lines in dose-dependent manner.
- Adiponectin suppressed malignant potential of human and mouse colon cancer cell lines.
- All these activation are mediated by STAT3/AMPK/S6 signaling pathways.
- Adiponectin regulated the expression of tumor suppressor and cell cycle regulatory genes.
- Despite the minor differences in magnitude of signaling activations, there are no major differences in malignant potential and signaling activation in response to adiponectin administration between human and mouse colon cancer cell lines.

Conclusions

- Exogenous administration of a physiological dose of adiponectin suppresses tumor growth.
- Adiponectin reduces the expression of angiogenic and proliferation markers.
- These effects are more pronounced in states of adiponectin deficiency, such as Western diet-induced obesity and metabolic dysfunction.
- Adiponectin directly controls malignant potential of the cells (cell proliferation, adhesion, invasion, colony formation) and regulates metabolic (AMPK/S6), inflammatory (STAT3/VEGF) and cell cycle (p21/p27/p53/cyclins) signaling pathways of colon cancer in LKB1-dependent way.

One Sentence Summary

These novel mechanistic studies utilizing the mouse model of obesity and metabolic dysfunction, which is closest to the obesity and metabolic syndrome induced by Western diet in humans, provide evidence for a causal role of adiponectin in colon cancer, suggesting that adiponectin could prove to be a useful agent in the management or chemoprevention of colon cancer.

Acknowledgements

In vitro
John Chamberland

In vivo
Xiaowen Liu
Kalliope Diakopoulos
Jutta Nagel

Technical assistance in the signaling study
Dimitrios Iliopoulos (Dana-Farber)

MCA38 mouse colon cancer cell lines
Nicholas Restifo (NCI)

1. Christos Mantzoros
2. Anastasia Koniaris
3. Konstantinos Aronis

4. Kyoung-Hee Park
5. Joo-Young Huh
6. Daria Lisicki

7. Bindiya Thakkar
8. Ayse Sahin-Efe
9. Ole-Petter Hamnvik

10. Kyoung-Eun Joung
11. Fadime Dincer
12. Lesya Zaichenko

13. Reena Berman
14. Kelsey Shields
15. Alexandra Tsolias

16. Maria Vamvini
17. Ertirea Mesfum
18. Holly Kilim

19. Natasha Gill

Thank you for your attention.