

Name: _____
(Please print your name)

**CANCER CELL SIGNALING
GS04 0133
MIDTERM EXAM
FEBRUARY 21, 2007**

The following exam consists of 11 questions. **You are to answer 7.** Please follow these guidelines:

You must answer:

- Question 3 or 4
- Question 6 or 7
- Question 8 or 9
- Question 10 or 11
- Any other 3 questions

You may then answer any other remaining questions until you have answered a total of 7 questions.

If you answer more than 7 questions, your grade will be the total calculation of the **lowest** scoring questions.

Please make certain your name is on the top of every page and that you check the appropriate box for submitting the test question answer for grading. Also, please be clear and concise with your answers.

Good Luck!

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 1: Dr. Mien-Chie Hung

Facts:

- a. The p21, a cell cycle inhibitor, is shown to be located in the nucleus to inhibit cell growth.
- b. Some clinical correlation studies reported p21 expression is high in malignant tumors, which is in contrast to the role of a cell cycle inhibitor.
- c. Assume you found an enzyme, which is known to be an oncogene that phosphorylates p21 and changes nuclear localization of p21 to cytoplasm.

Please make a hypothesis to link a, b. and c and design experiments to prove or disprove your hypothesis.

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 2: Dr. Gary Gallick

Cyclin-dependent kinases are important in promoting transitions through phases of the cell cycle. P27 is a Cdk inhibitor that binds Cdks (such as Cdk2) and inhibits its activity. The mechanism of inhibition of cell cycle progression by p27 is still not clearly understood. However, two very recent papers in the February 2007 issue of Cell (Chu et al. and Grimmier et al.) demonstrate that tyrosine phosphorylation by Src family members reduces the ability of p27 to inhibit Cdk2. With this in mind, design experiments that would address the issue of whether Src family phosphorylation of specific sites on p27 is required for cell cycle progression. The answer does not need to be specific; consider what approaches you would use and what results you might obtain. You do not need to worry about the precise roll of p27. Think about the Src problem.

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 3: Dr. Rakesh Kumar

Cell migration is important in physiologic and pathologic conditions such as cancer. Productive invasion requires reorganization of cytoskeleton and formation of leading edge, which in-turn translates into directional motility. The small GTPases such as Rac and Cdc42 and its effector kinase Pak1 have been implicated in the cellular motility and invasion of cancer cells. Under normal conditions, Pak1 kinase remains inactive and needs to be activated by cellular signals. Which one of the following statement is correct? Please explain why.

- A) Pak1 is activated by STATs and JAK kinases.
- B) Pak1 could be activated by selective small GTPase and proteins that interacts with the PBD domain.
- C) Pak1 exists in an open and active conformation.
- D) Pak1 activation requires its membrane targeting.

Point Value: 15 points

Name: _____
(Please print your name)

Submit answer for grading: yes no

Question 4: Dr. Rakesh Kumar

Ligand binding to the nuclear receptors promotes the recruitment of coregulators to the liganded nuclear receptor and binding of such complex to the NR response elements in the target genes. However, functionality of transcription-active is influenced by a fine balance of histone acetyltransferase (HAT) and histone deacetyltransferase (HDAC). Which one of the following statements is correct? Please explain why.

- A) Gene transcription by NR must involve methylation of surrounding histones.
- B) HDAC helps in promoting transcription from NR target genes.
- C) NR can not be activated by signaling kinase.
- D) Activated NR binds to the target promoters through specific response elements.

Question 5: Dr. Seth Corey

A 56 year-old man goes to your uncle, a physician, for tingling in his fingertips and forgetfulness. A blood count is performed, and it shows a hemoglobin of 69 (normal is 45). The physician suspects polycythemia vera (too many red blood cells). Your uncle knows that you are a graduate student at MD Anderson and he remembers reading about Jak2 mutations in last month's New England Journal of Medicine. Your uncle calls you and asks some questions.

1. Is the Jak2 mutation that causes polycythemia vera? Explain your answer.
 - a. gain-of-function
 - b. loss-of-function?

2. Since the tests came back and his patient doesn't have Jak2V617F mutation, your uncle asks you what else could it be due. Which one does not apply?
 - a. Loss of function mutation in SOCS3
 - b. A mutation in the kinase domain of Jak2 which prevents its ability to phosphorylate STAT5
 - c. A mutation in the Erythropoietin Receptor which causes Jak2 to dimerize without exposure to its ligand, erythropoietin
 - d. A mutation in STAT5 causing constitutive dimerization

3. Your uncle says he read about a new biotechnology company which is developing a drug which blocks the ubiquitinylation (proteasome breakdown) of STAT5 by SOCS3. Would this drug be of potential benefit to his patient? Yes or No.

4. The mRNA, obtained from the patient's bone marrow, has been made into cDNA, which is now sequenced using Jak2 primers. A mutation is found at codon 539, which results in a leucine substitution for lysine. Which of the following would not help you tell your uncle that this mutation is causing his patient's disease?
 - a. An in vitro kinase assay comparing Jak2K539L with wild-type Jak2.
 - b. Retroviral transduction of mouse bone marrow cells with Jak2K539L, which are then transplanted into a new mouse of the same strain.
 - c. Stable transfection of a leukemic cell line with Jak2K539L or wild-type Jak2, followed by tyrosine phosphorylation blotting of STAT5.
 - d. Knocking out the Jak2 gene in a mouse.

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 6: Dr. Ralph Arlinghaus

Your laboratory has just identified a new Bcr-Abl protein in chronic myeloid leukemia cells that is smaller in size in SDS polyacrylamide gels than P210 Bcr-Abl (210,000 mol. wt) and larger in size than P190/185 Bcr-Abl. It has an estimated size of 195,000 mol wt). You have cloned this new Bcr-Abl gene fusion as a cDNA and are able to express it in a normal cell line. You have found that RNA transcripts are produced in this cell. You know that P210 Bcr-Abl has a junction of either Bcr exon 13 or exon 14 joined to Abl exon 2 (e13a2 junction or e14a2 junction, respectively); the P190/185 Bcr-Abl has a junction Bcr exon 1 joined to Abl exon 2 (e1a2 junction). Knowing that you do not have the ability to sequence the entire mRNA that encodes the new Bcr-Abl oncoprotein and knowing that you are expert in PCR, what methods would you use in the lab to determine the junction point of Bcr and Abl exons in the new Bcr-Abl protein.

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 7: Dr. Ralph Arlinghaus

The c-Abl protein is a highly regulated protein tyrosine kinase in normal cells. When the kinase is inactive, it is known to be in a form of complex with two other components. One is PIP2 and the other is unknown and is termed "X". You have methods to isolate large amounts of the ternary complex, which contains c-Abl in an inactive state. What experiments would you perform to identify "X".

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 8: Dr. Bon Quy Trinh

a) Reinberg and colleagues identified the preinitiation complex (PIC) (Proc Natl Acad Sci U S A. 1991 November 15; 88(22): 9999–10003). Based on what you studied in class about PIC formation and the experimental design as below, draw a picture of expected results of the EMSA experiment and briefly explain the result on each lane (For simplicity, please assume that the concentration of general transcription factors and PolIII are just enough to form a complex and therefore no additional bands were found on the X-ray film)

Lane 1: probe only (Adenovirus major late promoter)

Lane 2: probe + TFIID

Lane 3: probe + TFIID + TFIIA

Lane 4: probe + TFIID + TFIIA + TFIIB

Lane 5: probe + TFIID + TFIIA + TFIIB + TFIIF

Lane 6: probe + TFIID + TFIIA + TFIIB + TFIIF + PolIII

Lane 7: probe + TFIID + TFIIA + TFIIB + TFIIF + PolIII + TFIIE

Lane 7: probe + TFIID + TFIIA + TFIIB + TFIIF + PolIII + TFIIE + TFIIH

Lane 8: probe + TFIIA + TFIIB + TFIIF + PolIII

Lane 9: probe + TFIID + TFIIA + TFIIF + PolIII

Lane 10: probe + TFIID + TFIIB + TFIIF + PolIII

b) What is the early checkpoint for correct promoter complex assembly? (One sentence should be enough)

Point Value: 15 points

Name: _____
(Please print your name)

Submit answer for grading: yes no

Question 9: Dr. Bon Quy Trinh

Cancer cells are smart! (or they are genetically and epigenetically unstable thus allowing rapid phenotypic changes in response to their environments). One of the processes by which these changes lead to metastasis is increased motility and invasion. Discuss how snail, a transcription factor, plays an important role in epithelial-mesenchymal transition (EMT) and some regulation mechanisms that influence on its activation, stability and localization.

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 10: Dr. Francois-Xavier Claret

You have recently characterized a novel transcription factor called *Fixolino* (Fix) as a nuclear oncogene. Fix can form homodimers and binds DNA to a specific response element called FRE (Fix-response element) on 5'-regularory region (promoter) of target genes. Fix is activated by phosphorylation through its upstream kinase called Fixk that phosphorylates Fix on two specific sites, Ser 72 and 78.

You hypothesis that Fix regulates negatively p53 at the transcriptional level **and have identified an FRE site on p53 promoter**. Design an experiment to show that Fix binds to and represses the p53 promoter.

Point Value: 15 points

Name: _____

(Please print your name)

Submit answer for grading: yes no

Question 11: Dr. Francois-Xavier Claret

Design an experiment to show that lack of Fix may not affect p53 expression through stabilization of protein.