Problem Set #3
7.06 - Spring 2015

Name _____

Section _____

Question 1
To test the TGF-Beta signal transduction pathway, you add TGF-Beta and look at distinct intervals after TGF-Beta addition. TGF-Beta is continuously present. Briefly describe the types of **molecular and cellular** changes and responses that you might observe at each of these different time points (assume that you are not limited by specific technical approaches). (2 pts each)

30 seconds
Ligand binding
Phosphorylation
SMAD localization (possibly)

60 minutes
Gene expression

24 hours
Differentiation/proliferation/etc. (change in cell state)

Question 2
The estrogen receptor pathway can respond to a signal with a single key component. In contrast, the Receptor Tyrosine Kinase pathway that is coupled to MAPK kinase signaling involves at least 8 different proteins. Based on the "core concepts" that we discussed for signaling pathways, what might be **three** advantages of having the additional complexity in the Receptor Tyrosine Kinase/MAPK pathway?

1. More steps allows for amplification of the signal
2. Modularity of pathway allows for crosstalk which can affect multiple downstream activities or respond to multiple upstream signals
3. More steps allows opportunities for feedback (positive/negative)

Question 3
In each case below, indicate whether the observation is an example of "negative feedback", "positive feedback", "crosstalk", or "amplification" of the signal transduction pathway. If the observation does not correspond to one of these, indicate "none".

A. The G protein coupled receptor associated with opsin is activated by each photon of light for 50 milliseconds. The time that it takes for a single G protein to bind, be activated, and release is 5 milliseconds.

Amplification

B. The RII kinase in the TGFβ pathway is always on, but only interacts with RI in the presence of ligand.

None

C. SMAD is a transcriptional activator that binds to TGFβ-responsive promoters. However, this DNA binding site overlaps (and is mutually exclusive) with a transcriptional repressor that is downstream of Ergosterol signaling.

Crosstalk

D. Epo treatment for the Jak/Stat pathway causes increased gene expression of the E3-ubiquitin ligase SOCS which modifies the cytokine receptor.

Negative Feedback

E. The MAP kinase downstream of Ras has 45 different potential substrates (i.e., Protein X, Protein Y, Protein Z, etc.). These proteins are not downstream of other signal transduction pathways, and a given MAP kinase activation event will only allow it to phosphorylate a single molecule of one substrate.

None

F. Recruitment of Grb2 to a receptor tyrosine kinase causes the dimerization of the receptor to persist for an increased amount of time.

Positive Feedback

Question 4
For each of the following, indicate whether the given mutation would likely cause the resulting protein to be non-functional, constitutively active, or dominant negative (Circle one)

A. A receptor tyrosine kinase lacking the transmembrane domain.

Non-functional / Constitutively active / Dominant Negative

B. A receptor tyrosine kinase lacking the cytoplasmic kinase domain

Non-functional / Constitutively active / ***Dominant Negative***

C. A receptor tyrosine kinase lacking the extracellular ligand binding domain

Non-functional / Constitutively active / Dominant Negative

D. A receptor tyrosine kinase where the extracellular domain can dimerize in the absence of ligand

Non-functional / ***Constitutively active*** / Dominant Negative

E. A receptor tyrosine kinase in which the tyrosine residues that are normally phosphorylated upon ligand addition are mutated to alanine residues.

Non-functional / Constitutively active / ***Dominant Negative***

F. A MAP kinase in which the residues that are normally phosphorylated by the MAP kinase kinase are mutated to aspartate.

Non-functional / ***Constitutively active*** / Dominant Negative

Question 5. For each of the mutants listed below, describe the ***specific molecular consequences*** of the mutation (or chromosomal translocation) on the function of the protein. In addition, in each case indicate whether the mutant will constitutively activate the pathway in the absence of a signal. (12 points).

1. A mutant that results in a truncated Raf lacking its Ras-associated regulatory domain.

   Specific change: ***This domain normally inhibits Raf activity. Removal of this domain would cause Raf to be active even if it did not associate with Ras***

   Constitutively active? ***Yes***

2. A MAP kinase kinase mutant that alters the site phosphorylated by Raf from serine to aspartate.

   Specific change: ***Aspartate mimics constitutive phosphorylation. This site is used to activate MAP kinase kinase during MAP kinase signaling, and thus would result in this constitutive activation***

   Constitutively active? ***Yes***

3. A mutant that changes the residues in the cytoplasmic domain of the Epo (Cytokine) Receptor surrounding the phosphorylation sites to random (but different) amino acids, but does not alter the specific residues in that domain that are phosphorylated by Jak.

   Specific change: ***Altering the surrounding sites would eliminate the consensus phosphorylation site. Even if the tyrosine residue remained, it would not be recognized by the kinase, and thus could not be active***

   Constitutively active? ***No***

4. A Gα mutant that is unable to undergo lipid modification.

   Specific change: ***This will prevent it from going to the membrane. Thus, it could not associate with the receptor and participate in G protein signaling***

   Constitutively active? ***No***

5. A chromosomal translocation that adds a transmembrane domain to NF-kB.

   Specific change: ***This would trap NF-kB on the membrane and prevent it from going to the nucleus. Thus, it could not activate transcription and would be inactive.***

   Constitutively active? ***No***

Question 6

You are trying to come up with an effective clinical treatment for forms of breast cancer where there is an increase in receptor tyrosine kinase signaling through a receptor named HerX. You have generated a series of monoclonal antibodies that have specific effects on the receptor. Which of these would you predict would be effective in treating the breast cancer *in patients*? Explain why or why not.

A. An antibody that binds to the receptor and alters the affinity of ligand binding from 10 nM to 10 μM.

This decreases the affinity of the receptor for the ligand, thus less receptor will be ligand bound and signaling will be decreases. EFFECTIVE

B. An antibody that binds to the kinase domain and inhibits kinase activity.

NOT EFFECTIVE. Antibodies are too large to enter the cell.

C. An antibody that induces dimerization of the receptor.

NOT EFFECTIVE. Inducing dimerization will artificially activate receptor signaling, Which is the opposite of what you want to achieve.

D. An antibody that induces endocytosis of the receptor.

EFFECTIVE. There will be less signaling molecules on the cell surface capable of signaling, thus overall signaling will be reduced.

E. An antibody that alters the dimerization specificity of the HerX receptor such that it will heterodimerize with a related receptor tyrosine kinase (HerY) that lacks phosphorylation sites for auto- or transphosphorylation and for SH2 binding.

EFFECTIVE. By forcing HerX to dimerize with HerY it wont be able to carry out any signaling functions, thus overall signaling will be reduced.