SP25 Group Activity #4 KEY

You are an expert studying the wound healing response. Recently, several of your colleagues have approached your group with some puzzling clinical cases related to wound healing abnormalities and are seeking your help to understand what is going wrong.

The first patient exhibits a very slow wound healing response, meaning the fibroblasts move toward the wound site, but at a reduced rate. A detailed investigation shows that their fibroblasts appear to be able to polymerize actin, but once in the F-actin form, they are unable to remove monomers from the filaments.

Q1. Why would the inability to depolymerize actin be an issue for fibroblast wound healing? (10 points)

Actin polymerization and depolymerization, known as treadmilling, allows cells to migrate in a particular direction via lamellipodia and filopodia extensions from the leading edge of the cell. This process involves the recycling of actin monomers from the minus end to the newly forming plus end to push out the cell membrane continuously. If unable to depolymerize actin, there would be no recycling or treadmilling, and new monomers of actin would need to be synthesized in order to push the leading edge, drastically slowing down the process.

Q2. What is likely the issue as to why the F-actin is unable to remove monomers from the filaments? (10 points)

There is likely an issue with the ability for F-actin to hydrolyze ATP, as the ADP-bound actin monomers are more weakly associated with the filament and therefore more likely to break off. Typically, upon joining the plus end of the filament, the ATP-bound actin monomer will undergo ATP hydrolysis to allow for treadmilling, which is not occurring here. It is also possible that there is some mutant accessory protein that is preventing the depolymerization of actin.

The fibroblasts of the second patient display no issues with migration, but instead appear to be unable to divide.

Q3. Which component of the cytoskeleton is likely affected in this patient? How do you know? (10 points)

The microtubules are likely affected here, as these are heavily involved in the cell division process as the principal component of the mitotic spindle, and during anaphase when the microtubules attach to the kinetochores of the chromosomes to allow for chromatid separation.

Deeper investigation reveals Patient #2 has a mutation in the protein MAP4, which prevents its dissociation from the cytoskeleton and promotes filament growth.

Q4. What effect does this mutation have on the dynamic instability of the cytoskeleton? Assuming that the "- end" of the filament is capped, what will occur at the "+ end" of the filament in these cells? (10 points)

As the minus end of the microtubule is capped and the MAP4 protein, a microtubule associated protein, remains bound and promotes filament growth, the addition of new α-/β-tubulin heterodimers will occur at a greater rate than the dissociation of GDP-bound dimers. This will lead to a net growth of the microtubule from the plus end with a large GTP cap.

The third patient's fibroblasts appear to have a fully functioning cytoskeleton, but are completely lacking Cyclin E.

Q5. What would you expect the outcome of this to be with regard to cellular function? (10 points)

Cyclin E is involved in the G_1/S transition of the cell cycle and its presence is required to bind to Cdk2 in order to progress through to S phase and DNA replication. If the cell cannot enter S phase, it will not divide, as so will remain in G_1/G_0 indefinitely.

Q6. Why is cyclin concentration so tightly regulated in normal cells? (10 points)

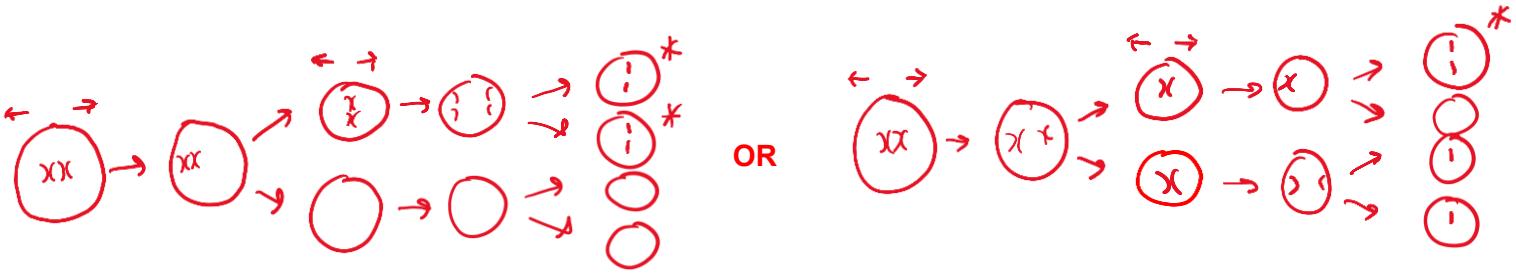
Cyclin concentration is so tightly regulated in normal cells because we do not want our cells replicating DNA and dividing unless everything is perfectly correct and appropriate. If conditions and not correct DNA replication could have caused mutations, the cell may be insufficiently large or not have the needed materials for cell division, or cell division may occur asymmetrically leading to mutant daughter cells. We synthesize cyclin only when it is needed after everything looks in good order, and then rapidly degrade it.

The next fibroblasts you look at come from a newborn baby. A karyotype analysis reveals an extra copy of Chromosome 10.

Q7. How is it possible for this baby to have 3 copies of Chromosome 10? Please draw a picture to accompany your response (20 points)

There was a nondisjunction event during Anaphase I or Anaphase II of meiosis, leading to asymmetric distribution of the chromosomes among daughter cells, which led to some haploid gametes having incorrect numbers of Chromosome 10.

X = Chromosome 10



Nondisjunction in Meiosis I **Nondisjunction in Meiosis II**

This baby has extreme thrombocytopenia, a very low platelet count, which interferes with their ability to form blood clots.

Q8. Provide two therapeutic approaches you could use to restore platelet counts in this baby. Why would these work? (20 points)

You could take fibroblasts from this patient and induce them into iPSCs, and then differentiate them into platelets *in vitro* and transplant these platelets than have the same DNA as the baby back into their bloodstream. As the cells are originally from the patient, they would not be immune-rejected by the host.

You could alternatively perform a bone marrow transplant, to provide a new source of functional hematopoietic stem cells, a multipotent progenitor lineage that gives rise to all of the blood cell

lineages, including platelets. This would likely require immuno-suppression to prevent the host from recognizing the bone marrow as foreign.

You could also differentiate iPSCs from the patient into hematopoietic stem cells and transplant these into the patient to avoid immune rejection, but if the Chromosome 10 aneuploidy prevented platelet differentiation originally, it may likely still prevent successful platelet production.