Answers

1 A) Yes
B) Adding EDTA will probably help to obtain single cells. Indeed, we know that the homophilic interactions between cadherins depend on Ca^{++}. Hence, chelating Ca^{++} ions will decrease their free concentration in the extracellular medium, causing a loss of cadherin-cadherin interactions and so a diminished cell-cell adhesion, increasing the possibilities to obtain isolated cells.

2 Answer: E. No negative impact because high [Ca^{++}] will have either no effect, or will reinforce the interactions (positive effect).
A) This will cause a decrease in cadherin transcription and therefore probably protein expression.

B) Trypsin will cause cleavage of extracellular proteins (after Lys and Arg residues) including cadherins.
C) Less cadherin membrane localization will decrease cell-cell adhesions.
D) Removal of the cytoplasmic part will impair the recruitment of intracellular anchor proteins and so the cytoskeletal filaments. This will perturb cell-cell adhesion. Additionally, often the stability of cadherins depends on their association with these anchor proteins, so cadherin degradation may occur.

3 A) The mutant E-cadherin proteins should still be able to interact with wild-type E-cadherin from group B cells via homophilic interactions, because their extracellular part is intact and they are still attached to the membrane. However, these mutant E-cadherins will be incapable of recruiting cytoskeletal filaments, because of the loss of the intracellular portion and hence incapacity to recruit the intracellular anchor proteins that make the bridge between cadherins and cytoskeletal filaments. A lack of cadherin-anchor protein complex usually results in cadherin instability and degradation. Hence, cell-cell adhesions between group A and B cells should be strongly impaired.

B) Mutant (hybrid) E-cadherin (intracellular + transmembrane) expressing the extracellular portion of Flamingo will not be able to make homophilic interactions with normal E-cadherin from group B cells anymore. We can imagine two different consequences: (1) cells of group B do not express Flamingo (no information on that has been provided) and so cell-cell adhesions between group A and B cells will be lost; (2) cells of group B express Flamingo. Because the cytoplasmic part of mutant E-cadherin is identical to wild-type E-cadherin, it is likely that recruitment of the intracellular anchor proteins will take place, enabling to recruit cytoskeletal filaments. Hence, one can predict (but experiments should be performed to test the prediction) that the cell-cell adhesions between group A and B cells are maintained.

4 Answer: 1. The inhibitor prevents the shedding of cadherin induced by the protease, and therefore facilitates cell–cell adhesion. This helps the cells clump together.

5 b-catenin is important for cell-cell junctions, specifically adherens junctions because it anchors cadherin proteins to the actin cytoskeleton. Additionally, b-catenin is important for intracellular signaling, where its nuclear accumulation will enable Wnt signaling to take place (see chapter Cell Communication and Signaling).

6 cAMP is a small molecule that can be shared through gap junctions between cells. In this case in the GPCR expressing cell, cAMP will have been made and have crossed the gap junction activating PKA and CREB in the cell that does not have the GPCR.

7 Fibrillar collagen is very rigid. Any of the collagens that are in that category are good to use. (I, III, V)

8: The Collagen IV containing ECM will resemble more the basal lamina. The ECM containing the network forming collagen IV will be much less rigid. Collagen I is a fibril forming collagens and will make the ECM stiffer.

9: The mutant fibronectin will not interact efficiently with the integrins; thus fibronectin will not be organized into focal adhesions. Also, within the cells the cytoskeleton will not be remodeled.
The cells will not look organized like in panel H, instead they will look more disorganized like panel A.

10A: Mutating the adaptor protein Talin will the most efficient. As all integrins depend on that protein to interact with the cytoskeleton. Potentially the talin recruiting protein RIAM would also be an option, however due to the presence of Talin some integrin-talin-actin interactions might still be possible.

10B: Vinculin mutation will disrupt the interaction between integrins and the actin cytoskeleton. However vinculin is also involved in adherens junctions, so it would not be a suitable choice to target integrins only.

10C: No as the interaction is lost between the integrins and the cytoskeleton, the cytoskeleton cannot be remodeled and cells will not move.

11: Hemidesmosomes are not involved as they interact with intermediate filaments, these are not easily remodeled like actin and tubulin cytoskeletal components. So the integrins in hemidesmosomes are not involved in migration, which depends on remodeling the actin cytoskeleton

12: Like most transmembrane proteins in eukaryotes integrins cross the plasma membrane with an alpha helix

13: These domains allow interaction between the different ECM proteins so they can build a network. If they would not interact the ECM would not be organized and would effectively fall apart.