**The cell cycle and cancer; liver as an exemplar – take home messages**

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***Question: How do tyrosine kinase-mediated signals trigger cell cycle entry from Go in mammalian cells, e.g. regenerating liver?***

Growth factors stimulate entry from Go into the G1 phase of the cell cycle via tyrosine kinase receptors, the Ras/Raf/ERK MAP kinase pathway, it’s action to stimulate the production of Myc which triggers cyclin D production, activation of Cdk4/6 and entry into G1.

***Question: How is the cyclical activation of the Cdks via pRb proteins achieved?***

***(Supplementary questions: Which Cdks are active in different stages of the cell cycle?***

***What sort of targets to Cdks have?)***

Cyclical activation of Cdks occurs by cyclin production and destruction. Each Cdk/cyclin complex stimulates the production of the next cyclin in the cycle, thus ensuring forward progression. Different cyclin/Cdk complexes act at different stages of the cell cycle. Positive phosphorylation by CAK kinase and dephosphorylation of the negative regulatory site by Cdc25 phosphatase are required for Cdk activation.

pRb forms inactive complexes with transcription factors of the E2F family. Cdks progressively hyperphosphorylate pRb causing the release of active DP/E2F transcription factors from pRb, thus providing one impetus to the transcription of genes needed for cell cycle progression.

Typical Cdk targets are proteins involved in DNA replication, nuclear breakdown and protein synthesis.

***Question: How does activation of the PI-3-kinase pathway occur and how does it affect the cell cycle?***

PI-3’-kinase phosphorylates PIP2 to PIP3 thus activating PDK1 and Akt/PKB. The PIP3 phosphatase PTEN opposes this.

***(Supplementary question: Overexpression of Akt has an anti-apoptotic effect in many cell types – why?***

Akt phosphorylates FOXO transcription factors which results in their exit from the nucleus blocking transcription of the negative cell cycle regulators p27Kip and apoptotic protein Fas ligand.

Bad binds and inactivates anti-apoptotic regulators Bcl-2 and Bcl-XL. Akt phosphorylates Bad to prevent this association.

Caspase 9 is a key protease in apoptosis - it is phosphorylated and inhibited by Akt.)

***Question: How do cell cycle inhibitors such as Cip/Kip and INK4 control Cdk activity?***

Cip/Kip and INK4 Cdk inhibitors regulate the cell cycle. The INK4 family inhibit Cdk4/6 in G1. On the other hand, p27Kip1 complexes with cyclinD-Cdk4 are active and promote cell cycle progression when unphosphorylated but are inactivated by phosphorylation promoted by GSK3b, which is itself inhibited by Akt/PKB – thus PI-3’-kinase promotes cell cycle progression by inactivating GSK3b thus maintaining active cyclinD-Cdk4.

***Question: How do cells check for DNA damage and what do they do if they detect it?***

p53 is a tumor suppressor protein that regulates the cell cycle and conserves genomic stability by preventing mutation.

* It can activate DNA repair proteins when DNA has sustained damage.
* It can induce growth arrest by holding the cell cycle at the G1/S regulation point on DNA damage recognition (if it holds the cell here for long enough, the DNA repair proteins will have time to fix the damage and the cell will be allowed to continue the cell cycle).
* It can initiate apoptosis if DNA damage proves to be irreparable.

p53 becomes activated in response to stress such as DNA damage, causing it to be stabilised and accumulate, as well becoming phosphorylated and activated as a transcription regulator. Kinases that can activate p53 include MAP kinases and ATR, ATM which are recruited and activated by DNA damage e.g. double-strand breaks. Activation of p53 leads to the activation of a number of genes whose products trigger cell-cycle arrest, apoptosis, or DNA repair.

***Question: Give examples of proteins controlling the cell cycle that are commonly mutated in cancers.***

***(Supplementary questions: Why does their mutation lead to a growth advantage?***

***Which proteins regulating the cell cycle might be good drug targets?)***

Oncogenes:

* EGFR/HER2, mutationally activated or overexpressed in many breast cancers
* Ras, mutationally activated in many cancers (>15%)
* CyclinD1, overexpressed in 50% of breast cancers
* B-Raf, mutationally activated in melanomas; v-Raf (deletion of regulatory domain)
* c-Myc, overexpressed in many tumours

Tumour suppressors:

* p53 – mutated in ~50% of human cancers
* PTEN (Phosphatase and tensin homologue deleted on Chr ten) - dephosphorylates phosphoinositides at 3’-position - loss of PTEN activates the PKB/Akt pathway, promotes cell survival.

- mutated in multiple advanced cancers - frequency similar to p53. Deletion in chromosomal region 10q22-25 observed in many cancers: prostate, renal, endometrial, melanoma, glioma, meningioma

* pRb, inactivated in many cancers
* p27KIP1, underexpression correlates with poor prognosis in many malignancies

Good drug targets:

* Cell surface proteins, e.g. growth factor receptor antagonists (antibodies such as Herceptin which inhibits HER2)
* Enzymes, e.g. kinase inhibitors
* Post-translational modifications, e.g. inhibitors of Ras lipid modification which attaches it to the plasma membrane