Module Code: BIO00008H

Examination Candidate Number: __________

Desk Number: __________

BSc and MSc Degree Examinations 2018-9

Department:
BIOLOGY

Title of Exam:
Cancer and the Cell Cycle

Time Allowed:
2 hours

Marking Scheme:
Total marks available for this paper: 100
The marks available for each question are indicated on the paper

Instructions:
Section A: Answer all questions in the spaces provided on the examination paper
Section B: Answer either question A or question B or question C. Write your answer on the separate paper provided and attach it to the back of the question paper using the treasury tag provided

Materials Supplied:
CALCULATOR
Module learning outcomes: Successful completion of this module will result in an understanding of, and an ability to synthesize arguments related to the following areas. Student will also be able to apply their knowledge to support evaluation of experimental evidence related to the following concepts in cancer biology.

1 The Hallmarks of cancer
2 The pathways that govern cell cycle commitment and progression, and their disruption in cancer cells
3 The concepts of tumour suppressors and oncogenes
4 DNA damage, repair and surveillance pathways that protect the genome
5 Nuclear organization and its disruption in cancer cells
6 The principles underlying the spread of cancers
7 Aberrant adult stem cell activity and its contribution to tumour formation
8 Current approaches in cancer research and their application
9 Modern approaches to cancer diagnosis and therapy, and the promise of personalized medicine.

Section A
Short answers 50 marks

Q1.

a) Outline three desired characteristics of a cancer biomarker. (3)

i)

ii)

iii)

· A molecular change that accurately reports on, or predicts, a feature of disease (presence, progression, response)
· Easily sampled, preferably in body fluids such as blood, sputum, urine ('liquid biopsy').
· Ideally it will also play a functional role, so be reporting directly on tumour biology (not essential)
· For diagnostic biomarkers, one that reports on an early change in tumour development, and so could detect individuals with pre-metastatic disease where curative surgical intervention is possible.

Any three points

b) Why are biomarkers important for patient stratification during evaluation of new therapeutics? (3 marks)

Modern treatments are often gene-focussed or mutated gene-focussed (1), so only by evaluating on the right patient group (1) can we know whether they work. The right patients are identified via molecular biomarkers, often reflecting expression of the drug target itself (1).

c) Outline the impact of accurate stratification on the evaluation of Gefitinib. (3 marks)

Any three of: The approved EGFR receptor inhibitor (1) is no more effective than placebo when evaluated on all NSCLC patients (1), but on those that are EGFR positive (1) it improves progression free survival time (1). Those that respond well have mutations in the tyrosine kinase domain (1) that increase sensitivity to the drug (1).
d) What do you consider to be the greatest barrier to widespread application of personalised medicine? (2 marks)

Students might say that knowledge generation leading to discovery of biomarkers and development of focused treatments is the greatest barrier (1), however a stronger answer is economics as we have plenty of effective molecules but few are adopted by state funded healthcare systems because greater impact can be achieved in other areas of healthcare (2).

Q1
a) This question was drawn primarily from the last lecture in the module and could be answered in many ways. Any answer that conveyed thought and understanding of the strengths and weaknesses of biomarkers, why we have so few useful cancer markers, and the problems faced during development, was credited. Many possible right answers.

b) Specifically relates to the message delivered in lecture 9 and the need for drug/marker combinations which most students articulated.

c) Specifically relates to the example study explained in lecture 9, though the term ‘stratification’ seems to have confused a few people even though it is in the slides.

d) I was looking for a thoughtful answer, not necessarily agreement with my point of view! This isn’t about learning information, but about thinking about the subject as a whole. Any sensible, articulated opinion was credited.

LO 1, 5, 6, 9

Q2. The images below show four mammalian cell nuclei from an unsynchronised cell culture that was exposed to two fluorescently labelled deoxynucleotide analogues during defined time windows; each 30 minutes long, separated by an hour. The two nucleotide analogues, one red one green, can be visualised independently in the same cell using fluorescent microscopy.

<table>
<thead>
<tr>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td></td>
<td></td>
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</tbody>
</table>

a) What phase of the cell cycle are these cells in and why? (1)

They must all be in S phase (0.5) because nucleotides only become incorporated into DNA when DNA synthesis is happening (0.5).

b) For cells b and c, explain why the red and green stains are not in the same place. (3)
The temporally separated labelling windows mean that different regions of DNA are captured in the process of replication and each replicates only once (1). Because DNA occupies preferred locations (1) in the nucleus (territories), and because DNA replication follows a sequential program (1) the DNA labelled in the two time windows is spatially separated.

c) Explain why in cell c there is a high concentration of green label around the edge of the nucleus. (3)

This is heterochromatin (1) that is replicating simultaneously within a narrow mid-late S phase time window (1). Some heterochromatin (generally not transcriptionally active) is preferentially concentrated near the nuclear lamina which is a repressive zone (1).

d) Which label were the cells exposed to first, red or green? Explain your answer. ? (3)

Red (1). Because cell a is only labelled in green and is displaying an early replication pattern (1) of well distributed small dispersed foci (euchromatin) - if red came later this cell would have both colours (1). Students might use cell d to illustrate their answer. This cell shows a late pattern in red, with no green. If green came before red the cell would be labelled with both colours.

e) Explain why a dark patch devoid of stain is evident in some cells. (1)

These are regions of the nucleus where there is no DNA, and are most likely nucleoli (the site of ribosome biosynthesis). Either point will get the mark.

Q2. This question tested ability to apply understanding of nuclear structure/function relationship, and was directly related to data on spatio-temporal organization of DNA replication explained in lecture 5. It was difficult, though the overall marks are quite good, and many people were able to apply their knowledge to work out the answers.
a) Most people realized that deoxynucleotide analogues are incorporated into newly synthesized DNA during S phase.

b) The relationship between location and time was grasped by most people, though a few people wrongly implied that the two analogues had different specificities (green-heterochromatin and red-euchromatin).

c) Most students understand that compacted, inactive, heterochromatin is normally peripherally located (also covered in stage 2 cell biology), though recognising it seems to have been a problem.

d) This was the difficult part as some people seemed to be confused about the two images of cell a (not grasping that it is the same cell), and in recognising its early S phase pattern. This alone is sufficient to say that red must come first, as outlined in the diagram.

e) Deduction that absence of DNA within the nucleus most likely defines the nucleolar region was achieved by most people, and relates to teaching (and images) of nucleolar distortion in tumour cells.

Q3.

a) Chromosome conformation capture (3C) can be used to ask whether two DNA sequences are located in close proximity within the nucleus. What do you consider to be the key steps in the methodology, and why? (5 marks).

Chemically cross-link sequences in close proximity to preserve the interaction (1), digest DNA so that cross-linked regions exist on linked short DNA molecules (1), ligate to create new molecules in which interacting sequences co-exist (1). PCR to identify the presence of specific sequence pairs or sequence whole samples for a genome-wide look (1). Computationally evaluate the frequency with which two sequences are connected (1). The technique can be applied over a temporal series of samples for questions about changing interactions during cell cycle or differentiation, and can be applied to single cells (1). Any 5 from above points.

b) At the level of the whole genome how might results of a 3C experiment differ between cancer and non-cancer cells? (2 marks)

A comparison would be expected to reveal less reproducible/lower strength interactions (1) within a cancer cell population, reflecting an unstable nuclear configuration (1).

c) Give two reasons why the same chromosomal translocations are frequently observed in tumours. (4 marks)

i) Templates in close proximity (for example those that are recruited to the same transcription or replication factory) are more likely to be mis-repaired to generate inappropriately connected fragments.

ii) Those translocations that give rise to a selective advantage will be detected more often because they contribute to acquisition of traits related to tumour formation.

Q3

a) This was well answered by most. However, extracting the key steps in this methodology was too much for some even though we covered it in lecture 4.

b) This question asked for students prediction/interpretation based on the teaching that relates to nuclear disorganisation touched on in multiple lectures. Generally answered OK.

c) This drew on understanding of double-strand break repair (L4) and knowledge of common translocation events in cancers.

LO 2, 5, 8
Q4.

a) Explain how DNA damage induced by ultraviolet light is converted into a mutation. (3 marks)

Upon passage of the replication fork/DNA replication (1), mis-recognition of damaged nucleotides by DNA polymerases can result in incorporation of the wrong nucleotide in the daughter strand (1), creating a mismatch. Only after a second round of replication (1) is a daughter cell with a stable point mutation (1) created. Any three points that conveys the meaning

b) Suggest two situations in which DNA repair pathways cannot maintain 100% fidelity of daughter cell genomes. (2)

i)

ii)

Any two of

i) Spontaneous decay leading to occurrence of occasional abasic sites, or other unrecognisable nucleotide distortions.

ii) Overwhelming levels of damage, such as after chemotherapy or occupational exposure to carcinogens

iii) Compromised repair or checkpoint pathways due to acquired or inherited mutation of genes encoding repair enzymes.

c) Outline the two main pathways that deal with sequence errors introduced by inaccurate DNA replication. (4)

i) Proof-reading subunits of DNA polymerases (1) typically reduce error rate by 100 fold (1) by detecting, removing and replacing incorrect bases (1) at the replication fork (1).

ii) Mismatch repair enzymes (1) reduce error rate by a further 100 fold (1). They detect structural distortions created by mis-paired bases (1) and remove a short stretch including the error (1).

Any combination of two points are available for each part.

Q4  a) This was answered well on the whole, with most people conveying the key point that passage of the replication fork is important. It was not always expressed explicitly though and some people continue to confuse damage with mutation.
b) Some people listed mutations in two different types of repair pathway, which only scored one mark. In addition to the answers above, a mark was awarded for NHEJ, which is error prone under normal circumstances.

c) The most common error was to talk about pathways that recognise damage (spontaneous or induced), rather than mis-incorporated undamaged nucleotides as the question asked. Relates to lecture 3.

LO 1, 3, 4

Q5.

a) At least three of the cell types in prostate cancers are known to harbour gene rearrangements. Outline the cell types, the rearrangements and their effect on tumour homeostasis in the table below.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Rearrangement</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer (epithelial cells)</td>
<td>TMPRSS2-ERG</td>
<td>ERG oncogene activation, genomic instability</td>
</tr>
<tr>
<td>B Cells</td>
<td>Immunoglobulin Genes</td>
<td>Immune responses against onco-antigens</td>
</tr>
<tr>
<td>T cells</td>
<td>T cell antigen receptor genes</td>
<td>Immunorepressive responses to maintain tumour immunity</td>
</tr>
</tbody>
</table>

Any 6 points

b) In order to study gene expression patterns and hence the functions of the individual cell types in a tumour it is sometimes necessary to purify specific populations. Why would the gene expression results (by RNAseq or gene expression microarray) from a total homogenate of a cell mixture differ from the sums of the individual cell types? (2 marks).

Most RNA seq and expression array experiments require the prior amplification (0.5) of nucleic acids (RNA/DNA) from the tumour. Minor proportions within the cell mixture (less than 10%) of the mass are not amplified to the same extent and disappear from standard analyses (0.5). Highly secretory cancer cells dominate any expression signatures, relative to quiescent stem-like cells (0.5). With a single cell analysis this does not happen, but then you have to assign a phenotype to the cells based on their expression pattern, unless you fractionate according to a known cell surface phenotype first (0.5).

Q5

a) This question was not answered well. The full correct answer required some lateral thought about the heterogeneity of real cancers in man, compared to cancer cell lines and was based on material taught in lectures (on the lecture slides and reinforced in the lecture itself). The simple answer to the gene rearrangement question (NOT gene expression) was the TMPRSS2-ERG gene fusion in prostate cancer epithelial cells which drives stem cell division. The other two
rearrangements were in the infiltrating T and B lymphocytes which I said indicated an active immune response within tumours (being harnessed for immunotherapy).

b) This was a more technical question about the effects of heterogeneity, and could be answered on two levels. The first relates to the presence of many cell types, which can swamp the expression of genes from cells present in smaller numbers such as stem/progenitors (relates to heterogeneity). Most students got this part. The second part of a full answer concerned the PCR process. You were given a diagram and an account of amplification kinetics in PCR (and RT-PCR/RNA Seq) which showed a threshold effect below which an RNA would be deemed undetectable. The argument works for both lowly expressed genes in all cells and also highly expressed genes in a small % of the tumour mass.

LO 1, 7, 8

Section B

Essays 50 marks

A. Breast cancer metastasis to the brain is rare, and symptoms may not present until decades after resection of the primary tumour. A possible explanation is dependence on reprogramming of, and adaptation to, the neural niche. Design and discuss an experimental approach to test this hypothesis.

A model answer will start by generating an appropriate experimental system that allows you to look at breast cancer to brain metastasis. For example, by using murine xenografts of brain-homing breast cancer cells (e.g. 231-brain). Alternatively, a transgenic mouse model could be used. To study the dependence of brain metastasis on reprogramming the neural niche, a first step would be to identify cellular and molecular changes occurring at the metastatic site before, during and after metastasis, e.g. via imaging or biochemical approaches (intravital 2P microscopy, western blotting, LCMS). Adaptation, by contrast, is a process intrinsic to the tumour cells themselves and could be studied in situ using similar approaches or by ex vivo metabolic profiling. Candidate factors/metabolites/heterotypic signalling events could then be studied in detail using genetic (overexpression or KO) approaches and effect on tumour latency evaluated. Consideration should be given to the choice of appropriate controls and analytic approaches. Discussion should focus on appropriateness of the model to study late stages of the metastatic cascade that relate to the secondary site, including extravasation, latency/dormancy, fate of micrometastases, and the heterotypic contribution of the metastatic niche.

LO 1, 6, 8, 9.
There was a wide range in the quality of answers to this question. In general, none of the essays covered all points in the model answer. However, there were several additional and alternative approaches that were proposed, some of which were insightful and likely to give good data. One of the biggest omissions was to focus only on adaptation (acquisition of GABAergic phenotype was the most common example cited) but to ignore the other part of the question on reprogramming of the neural niche. Some, however, addressed the latter very well, hypothesising that LOX priming might occur and describing various strategies to test this.

B. With focus on events that occur downstream of the mammalian restriction point, explain how multiple regulatory mechanisms cooperate to ensure that the cell cycle is a one-way series of events.

This essay will focus on the concept of commitment to a new cell cycle upon passage through the restriction point (R). It will used examples to illustrate that the cell cycle cannot go backwards because it is governed by sequential expression, activation and destruction of cyclins, emphasizing that their expression is dependent on the one before. It will touch on events upstream (regulation of cyclin D-CDK4), but focus on events that are downstream (activation of cyclins E/A-CDK2), and subsequent effects on expression of genes involved in Cell cycle progression, Replication complex assembly,
DNA synthesis, DNA surveillance, DNA repair, Chromatin structure, Mitosis (mentioning some example factors eg Cyclin E, P27, Cdc6, Mcm3, DNA pol a, Chk1, P53, MutS, Brca1, Histone 2A, Securin). It will include an explanation of the pivotal role of the retinoblastoma protein itself, and how the restriction point is a one way switch. It will talk about how R is governed by CDK-dependent phosphorylation of Rb, triggered first by cyclin D and then by sudden build-up of cyclin E via a positive feedback loop, and how Rb status modulates the function of the E2F family of transcription factors. A complete essay will also make reference to the proteasome-mediated destruction of the Cdk-inhibitor p27, which normally restrains the activity of CDK2 downstream of R. At the same time as phosphorylation of Rb, cyclin E-cdk2 also phosphorylates p27 which promotes its destruction by proteasomal degradation. In the absence of p27 the cell cannot re-establish a restrained state as P27 is not resynthesized until the next cell cycle. For additional marks the essay, could also make reference to viral proteins that intervene in the Rb/E2F axis, and/or to the human genetics of Rb inheritance and its classification as a tumour suppressor gene and relationship with childhood retinoblastoma. Throughout it will acknowledge that most of the players in G1 control are mutated in some types of cancer.

Replication complex assembly and activation (post-R) occur within temporally separated, mutually exclusive time windows that are ordered by dependence of cyclin expression (A on E), and by concentration dependent events along a rising gradient of cyclin expression (A). E/k2 cooperates with Cdc6 to assemble MCM helicase at replication origins to create pre-replication complex. E/K2 leads to expression of A (via Rb/E2f axis) creating dependency. Low A/K2 phosphorylates and inactivates Cdc6 (export and/or degradation). Higher A/k2 supports loading of polymerase to create activated replication complex. Also phosphorylates and displaces MCM, which cannot be reloaded until Cdc6 is available again in next G1. This is a mechanism to prevent re-initiation of DNA replication.

This question was dealt with very well. Almost everyone who chose to answer it focussed on the right regulatory loops with varying degrees of detail and accuracy. In addition to the cyclin E relationship, there was appropriate inclusion of the mechanisms that order replication complex assembly and activation at G1/S. The best answers linked the information to cancer biology. We also looked for answers that highlighted the forward drive rather than mechanisms for arrest.

LO 1, 2, 3.

C. Evaluate hypotheses to explain the development of treatment-resistant cell populations and suggest a strategy to overcome such resistance based on your knowledge of tumour cell biology. Wherever possible use specific examples of actual treatments and tumour types to support your arguments.

A good essay will cover the relative importance and evidence for the presence of pre-existing treatment resistant cells (stem-like) within the tumor mass. These cells then grow and evolve to produce new tumor cell clones with an increased fitness to survive in the post-treatment microenvironment.

The opposing hypothesis is that cancer resistance is induced by the treatment, probably due to a genomic instability and random chance emergence of a resistant clone, which then dominates the new resistant tumour cell phenotype. This is also known as de-differentiation or transdifferentiation.

The evidence for both is difficult to gather from real cancers, but the stem cell hypothesis is supported by studies of primary cell cultures and xenografts from cancers, whilst transdifferentiation is mainly seen in cell lines studies, in vitro and in vivo.

In both cases the cancer would share some of its core/trunk mutations with the relapse so deciding which mechanism is preferred (both may occur depending on the treatment used). A discussion of such mutations is a good scoring point.
The emergence of a new clone from a primitive progenitor is argued for by the absence of some treatment naïve mutations which give the early tumor a growth advantage in the resistant population. e.g. IDH1 andTMPRSS2-ERG in hormone resistant metastatic prostate cancers...AND/OR how can a mutant emerge in the presence of mitotic inhibitors, which will require cell division, unless there are pockets of a cancer with sub-optimal concentrations?

Do defects in DNA repair genes (found in resistant relapsed cancers) accelerate the development of the resistant cells? These changes are MUCH rarer in the untreated cells!

A mention of epigenetic control as a flexible means of silencing genes under a tumor treatment regime with ready reversal later would also score well.

Stem cell resistance mechanisms such as quiescence, Drug pumps (ABC transporters), inherent radiation protection and repair such as chromatin condensation, rapid assembly of repair complexes (SMARCps) would be additional and good scoring details.

Amplification and mutation of the SINGLE COPY of the androgen receptor gene provides the easiest route to resistance against agents which target both the supply of androgens and its binding to the androgen receptor. This is similar to methotrexate resistance with DHFR gene amplification.

Hopefully, a first class essay will bring in information from other lectures perhaps including immunotherapy, which is very topical?

I (NJM) provide most information about prostate cancers so I would expect the answers to be biased towards this.

The essay was designed to elicit ideas about the various (not just Stem Cell) based ideas about tumor cell resistance to treatments. In general it was really well answered with a much higher average mark than I normally would expect. I was delighted that the students grasped this important output from the lecture, though more students do need to think of specific examples (which I said would get extra marks).

LO 1, 7, 8