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Review Document

Lecture 1 amino acids, pH and pKa

1. Be familiar with properties of amino acids, which side chains act as acids or bases, the charge of the amino acid side chains.
2. Property of peptide bonds, no free rotation due to partial double bond character.
3. Cysteine and cystine.
4. Calculations from Henderson-Hasselbalch equation. Know effect on ratio of base to conjugate acid when pH is equal to pK_a and when pH and pK_a differ by integer units.
5. Concept of pI and protein charge. Be able to interpret electrophoresis and isoelectric focusing data.

Lecture 2, the three dimensional structure of proteins.

1. Phi and psi angles and generation of secondary structure.
2. Characteristics of alpha helical and beta structures.
3. Motifs, folds, domains, super-folds.
4. Dynamic nature of folded proteins.
5. Different properties of fibrous proteins vs. globular proteins. Structures of collagen, keratin and myosin as examples of fibrous proteins.

Lecture 3. Hemoglobin.

1. Tetramer structure, heme, iron oxidation state, oxygen binding site, T and R conformations
2. Effect of hydrogen ion (pH) and DPG on the equilibrium.
3. Effect of hydrogen ion (pH) and DPG on the P_{50} value. Meaning of P_{50} value.
4. Role of hemoglobin in carbon dioxide transport (isohydric and carbamino-Hb).
5. Interpretation of the Hill equation.
6. Sigmoid curves shown in cooperativity in substrate binding.-
7. Effect of mutant hemoglobins, HbS mutation (beta chain 6Glu->Val), prec. of HbS in the T conformation.

Lecture 4.Enzyme Kinetics.

1. Michaelis-Menten equation, meaning of velocity, K_m, k_{cat}, V_{max}. Dependency or lack of dependency of a parameter on E and S.
2. Interpretation of Lineweaver-Burke equation plots and v vs. S plots.
3. Types of 2 substrate reactions (ping-pong, ordered binding, random binding).
4. Competitive and non-competitive inhibition. Changes in $K_{m(obs)}$ and $V_{max(obs)}$. Changes in v vs. S and Lineweaver-Burke in presence of inhibitors. Meaning of K_I.
5. Properties of rate controlling enzymes in metabolic pathways.

6. Meaning of the Gibbs energy changes related to the energy of activation (effect on rate constant) and the energy of activation for reaction (effect on equilibrium constant). What is the effect of an enzyme on these relationships?

Lecture 5. Regulation of gene expression 1. The components,
1. The gene unit (promoter region definition, regulatory elements in the gene unit (distance and orientation dependency).
2. Regulation of chromatin structure by HAT and HDAC enzymes (histone acetylation and deacetylation) and by chromatin remodeling enzymes (SWI/SNF type enzymes). Describe the role of the lysine ammonium group in forming condensed chromatin and the role of acetylation in this mechanism. Methylation of histone proteins (histone protein methylation leads to condensed nucleosome structure).
3. Common structures of transcription factors (recognize a palindrome for binding of a dimer transcription factor), helix-turn-helix motif, and helix-loop-helix, zinc finger, and bZIP proteins. Role of leucine zippers.
4. Interpret a DnaseI foot print assay, gel retardation assay, and a regulatory sequence reporter gene (i.e. firefly luciferase, CAT or beta-galactosidase) assay.

Lecture 6. Regulation of gene expression 2. Mechanisms.
1. Combinatorial regulation of gene expression leading to cell division or differentiation through Myc/Max/Mad
2. Kinases phosphorylate c-Jun and c-Fos, activating them as transcription factors.
3. Mechanism of X-chromosome inactivation in females, mosaic expression. Also mechanism of the LCR region regulating beta-globin gene expressions in erythrocytes.
4. Epigenetic effects, definition. Epigenetic information transmitted through positive feedback loop with transcription factor genes.
5. DNA methylation, usually turns off gene expressions. CpG methylation site. CpG islands in housekeeping genes are not methylated. Maintenance methylase acts to fully methylate hemimethylated sites in progeny cells.
6. Gene imprinting carried out by methylation.
7. Maintenance acetylases preserve sites of histone acetylation and euchromatin or heterochromatin in progeny cells.

Lecture 7. Cell cycle 1.
1. Mechanism of Cdk activations: cyclin synthesis and degradation, CAKs, Wee1 inhibition, CDC25 phosphatase activation.
2. Role of Rb and E2F through cell cycle.
3. p53 and p21 regulation of cell cycle.
4. Effect of viral oncogenes on Rb and p53. Viral regulation of cell cycle.
5. APC and SCF ubiquitin systems regulation of cell cycle.

Lecture 8. Cell cycle II.

1. Growth factor pathways, receptor dimerization, tyrosine kinase activity, Grb-2 (adaptor protein), functions and specificity of SH2 domains and SH3 domains. Ras-GEF, Ras, MAPK pathway. Activating (oncogenic) mutations in Ras. Role of Ras-GAP.

2. Apoptosis. Apoptotic inducers, role of Bcl-2, caspases, viral gene products (CrmA, p35 (a Bcl-2 homologue)), Bax and Bak (anti-apoptotic), Bad and Bid (facilitators of apoptosis), T-cell mechanisms (granzyme, Fas ligand). Difference between apoptotic death and necrotic death. One or two sentence explanations of the role of apoptosis in cancer, AIDS, neurodegenerative disorders, ischemic disease, viral infection, autoimmune disease.

3. Definition of proto-oncogene and tumor suppressor genes. Identify which is which in cell cycle and apoptotic pathways.

Lecture 9. Cancer 1.

1. Definition and some description of the concept or properties of cellular immortality, angiogenesis, metastasis.
2. In apoptosis, identify properties death receptor pathway (Fas receptor, adaptor proteins, caspase 8); and mitochondrial pathway (cyt. C, Apaf1, apoptosome, caspase 9, Bak, Bax, Bad, and Bcl-2). Different activators of apoptosis, p53 acts by increasing concentrations of Bad-like proteins relative to Bcl-2-like proteins.
3. Role of Ras pathway in regulating apoptosis (not specific MAPKs involved).
4. p53 regulation through mdm-2 and ubiquitin pathway, Arf, DNA kinases.
5. Metastasis: uPA, procollagenase type IV, PAIs, TIMPs. Three step mechanism of metastasis. Role of laminin receptors.
6. Angiogenesis: FGF and VEGF, effect on endothelial cells (invasion through increased secretion of uPA and procollagenase and decreased secretion of PAIs and TIMPs). Role and source of angiostatin and endostatin.

Lecture 10. Cancer 2.
1. Single mutation is not sufficient.
2. Cancer is derived from a single abnormal cell but cells in a tumor are heterogeneic.
3. Carcinogenic mechanisms (initiators (mutagens) and promoters). Effect of diet and environment (smoking) on cancers.
4. Cancers are derived from activating a protooncogene (dominant mutation) or inactivation of both alleles of a tumor suppressor gene (recessive mutations). Know examples of protonocogenes and tumor suppressor genes. Chronic myelogenous leukemia (CML, Philadelphia chromosome) due to activation of Abl, a Src-like tyrosine kinase. Inhibition of Abl tyrosine kinase activity with a drug inhibitor cures CML.
5. Onocogenic viruses can cause cancer through inhibition of Rb and p53.
6. Example of cholorectal cancer. 50-60% of colorectal cancers show loss of APC gene, p53 gene, activation of ras gene during the carcinogenic process.
7. Different cancers have activation of different proto-oncogenes and loss of tumor suppressor genes. Can be analyzed with a cDNA array or proteomics. Some common overlaps usually present (p53 mutated in 50% of cancers; and effects on p53 due to ARF

and mdm-2 dysregulation in other cancers results in p53 malfunctioning in most cancers. Rb gene regulation is a common target as is Ras (mutated in 30% of cancers). Bcl-2 is often overexpressed. Inhibition of apoptosis by loss of p53 or increase in Bcl-2 also leads to resistance of many cancer cells to anti-cancer drugs.