

- The inflammatory cytokine TNF is directly transforming in vitro
- The long term use of nonsteroidal anti-inflammatory drugs decreases mortality from colorectal cancer
- Selective COX-2 inhibitors reduce cancer incidence
- Polymorphisms in genes that regulate immune balance influence cancer risk
- In cancers, an abundance of infiltrating innate immune cells, such as macrophages, mast cells, and neutrophils, correlates with increased angiogenesis and/or poor prognosis
- In cancers, an abundance of infiltrating lymphocytes correlates with favourable prognosis

Inflammation dependent tumour promotion

In a mouse tumour model, liver carcinogenesis was provoked through deletion of the *mdr* (multidrug-resistance) gene in the mouse germ line, loss of this gene and its encoded product leads to accumulation of bile acids in the liver and resulting chronic liver inflammation. Affected mice develop nodules of dysplastic hepatocytes, localised hepatocellular carcinomas (HCCs) and eventually metastatic HCCs. In the liver of these mice, TNF- α is initially produced by inflamed endothelial cells as well as infiltrating immune cells in the stroma, such as neutrophils and macrophages. The released TNF- α acts in a paracrine manner on nearby hepatocytes, which display receptors for TNF- α . The TNF- receptors respond by activating IKK, which induces NF- κ B signalling, which drives expression of anti-apoptotic genes (eg. Bcl-XL), proliferative genes (eg. cyclin D1 encoding genes), and the gene encoding TNF- α , expression of additional TNF- α leads to amplification of the inflammatory response. Together, the proteins encoded by these genes facilitate the progression of the dysplastic nodules to hepatocellular carcinomas. This progression can be blocked by antibodies reactive with TNF- α as well as a dominant-negative, non-inducible I κ B. When NF- κ B signaling was blocked in the hepatocytes of the *mdr*- mice, through the introduction of an anti-TNF- α antibody which blocked the paracrine I κ B in the hepatocytes which inhibited NF- κ B activation, tumour incidence was strongly suppressed.

Chronic inflammation plays a role in the pathogenesis of human carcinomas. HCCs, which are common in East Asia, are associated with chronic hepatitis B virus (HBV) infections and accompanying inflammation of the liver. HBV may create liver cancer through its ability to cause continuous cell proliferation in an organ that normally experiences very little, this proliferation is required to replace hepatocytes that are continually being killed by HBV infectious cycles. Alternatively, HBV infection may cause cells of the immune system to attempt to eliminate virus-infected cells, yielding a chronic inflammatory state in the liver. Chronic hepatitis C virus infections act in a similar way to increase liver cancer rates.

Genome-wide association studies have identified several inflammatory bowel disease (IBD) susceptibility loci that contain genes that encode cytokines and proteins that involve cytokine signalling. Loss-of-function mutations in the genes encoding IL-10 and the IL-10 receptor are associated with very early onset IBD cytokines not only drive intestinal inflammation and diarrhoea in IBD but may also regulate extra-intestinal disease manifestations and systemic effects. Cytokines have a role in driving complications in IBD such as intestinal stenosis,

antioxidants either block the formation of free radicals or scavenge them once they have formed. In addition, free radicals are inherently unstable and decay spontaneously.

The generation of free radicals is increased and the activity of protective endogenous antioxidant enzymes decreases, in several situations; the absorption of radiant energy (such as UV light, or X-ray), the enzymatic metabolism of exogenous chemicals (such as drugs), inflammation, and in diabetes. These situations result in high amounts of superoxide anion being produced, overwhelming antioxidant enzyme capabilities, thus hydrogen peroxide is converted to the highly reactive hydroxyl radical, instead of being broken down.

ROS, such as hydroxyl radicals, cause cell injury by three mechanisms; lipid peroxidation of membranes, double bonds in membrane polyunsaturated lipids are vulnerable to attack by ROS, the lipid radical interactions yield peroxidases, which are themselves unstable; cross-linking and other changes in proteins, free radicals promote sulfhydryl-mediated protein cross-linking, resulting in degradation or loss of enzymatic activity; DNA damage, free radical reactions with thymine in DNA produce single-strand breaks.

The kidney

The most superficial region of the kidney is the renal cortex. Deep to the cortex is the renal inner medulla. The cortex is composed of nephrons. The medulla exhibits renal pyramids. The pyramids are formed of parallel bundles of urine collecting tubules and capillaries. The pyramids apex are the renal papilla to drain urine from the collecting ducts via papillary ducts into the minor calyx. Nephrons are the structural and functional units of the kidneys. The nephron regulates excretion by; filtration, glomerular filtration takes place at the glomerulus and produces a cell and protein free filtrate; reabsorption, tubular reabsorption is the process of selectively moving substances from the filtrate back into the blood, reabsorption takes place at the proximal convoluted tubule and the collecting duct; secretion, tubular secretion is the process of selectively moving substances from the blood into the filtrate, it takes place along the proximal convoluted tubule and collecting duct.

The loop of Henle is responsible for concentration of urine, through the action of countercurrent mechanisms. The exchange of substances between the thin descending limb and thick ascending limb ensures efficient reabsorption of solutes and water and establishes a osmotic gradient in the peritubular fluid that permits passive reabsorption of water from the tubular fluid in the collecting duct. The kidneys have a high oxygen concentration due to transport, as such the kidneys receive 20% of the cardiac output.

The functions of the kidney include; excretion of endogenous waste products, excretion of drugs and their metabolites, water and electrolyte balance, acid base balance, and production of hormones such as erythropoietin and renin.

Kidney disease

In kidney disease there is; a reduction in renal excretory function (uraemia, and drug toxicity result), an inability to maintain water-electrolyte balance and acid-base balance (metabolic consequences result), and reduced hormone function (anaemia and hypertension result). Renal failure may be rapid, acute kidney injury (AKI), or may take many months or years to

cleavage step, which removes the terminating/inhibiting group and the fluorescent dye. Additional Washing is performed before starting the next incorporation step. Helicos Biosciences uses a one colour-CRT cycle. Illumina- Solexa uses a four-coloured CRT cycle.

Sequencing by ligation (SBL) is another cyclic method that differs from CRT in its use of DNA ligase and either one-base encoded probes or two-base-encoded probes. A fluorescently labelled probe hybridized so its complementary sequence adjacent to the primed template. DNA ligase is then added to join the dye-labelled probe to the primer. Non-ligated probes are washed away, followed by fluorescence imaging to determine the identity of the ligated probe. The cycle can be repeated either by using cleavable probes to remove the fluorescent dye and regenerate a 5' phosphate group for subsequent ligation cycles, or by removing and hybridizing a new primer to the template.

Pyrosequencing is a non-electrophoretic, bioluminescence method that measures the release of inorganic pyrophosphate by proportionally converting it into visible light using a series of enzymatic reactions. Pyrosequencing manipulates DNA polymerase by the single addition of a dNTP in limiting amounts. Upon incorporation of the complementary dNTP, DNA polymerase extends the primer and pauses DNA synthesis is initiated following the addition of the next complementary dNTP in the dispensing cycle. The order and intensity of light peaks are recorded as flowgrams, which reveal the underlying DNA sequence. Pyrosequencing is utilised by Roche-454.

Real time sequencing involves imaging the continuous incorporation of dye labelled nucleotides during DNA synthesis. Single DNA polymerase molecules are attached to the bottom surface of individual zero-mode waveguide detectors that can obtain sequence information while phospho linked nucleotides are being incorporated into the growing primer strand. Alternatively, DNA polymerase with an attached fluorescent dye can be used, that upon incorporation of their gamma-labeled nucleotides produce an enhanced signal by fluorescence resonance energy transfer.

Pharmacogenomics

In pharmacogenomics, genomic information is used to study an individual's responses to drugs. When a gene variant is associated with a particular drug response in a patient, there is the potential for making clinical decisions based on genetics by adjusting the dosage for choosing a different drug. Multigene analysis of whole genome SNP profiles can be used to assess gene variants affecting an individual's drug response. Pharmacokinetics and pharmacodynamics determine drug action in individuals. Pharmacokinetics encompasses four processes; absorption (how a drug enters the bloodstream), distribution (where the drug travels after absorption and how much drug reaches the target site), metabolism (how the drug is broken down in the body), and excretion (how the drug leaves the body).

Pharmacodynamics is the molecular action of a drug on its target. Genetic variation in genes encoding drug-metabolising enzymes, drug receptors, and drug transporters have been associated with individual variability in the efficacy and toxicity of drugs. Genetics also underlies hypersensitivity reactions in patients who are allergic to certain drugs. Predicting serious adverse drug reactions is a priority for pharmacogenomic research. For example, the enzyme CYP2D6, one of a class of drug-metabolising enzymes found in the liver, breaks

an electrical charge, and can be deflected into a separate pot for further analysis. The flow cytometer can be used to measure T-cell subpopulations, an important diagnostic indicator in AIDS. when the number of CD4+ T-cells in the blood of a patient with AIDS falls below a certain level, the patient is at high risk for opportunistic infections.

ELISA

Enzyme linked immunosorbent assays use antibodies covalently bound to enzymes, the conjugated enzymes are selected on the basis of their ability to catalyze the conversion of a substrate into a coloured-fluorescent, or chemiluminescent product. ELISAs can be used qualitatively to detect the presence of antibody or antigen, alternatively ,a standard curve based on known concentration of a sample. ELISA applications; to quantify serum antibody concentration against HIV, TB, to detect hepatitis B markers in serum, biomarker monitoring for example prostate specific antigen, and to detect allergens in food products.

Biomarker- a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.

Prostate specific antigen as a biomarker for prostate cancer

Prostate specific antigen (PSA) is a single chain glycoprotein with a molecular weight of 34kDa. PSA is secreted by the prostate epithelium in normal, benign, and cancerous conditions. ELISA can detect and quantify the level of PSA in patient serum. PSA levels can be used to monitor the progression of prostate cancer. Additionally, PSA levels can be used in conjunction with a digital rectal exam to test asymptomatic men for prostate cancer. PSA serum levels are elevated in patients with prostate cancer, benign prostatic hypertrophy and inflammatory conditions, such as prostatitis. 5% of men with a PSA level less than 4 ng/ml go on to develop prostate cancer. 31% of men with PSA levels 4-10 ng/ml go on to develop prostate cancer. 50-65% of men with PSA levels above 10ng/ml develop prostate cancer.

Mass spectroscopy

Mass spectroscopy is an analytical tool useful for measuring the mass-to-charge ratio of one or more molecules present in a sample. MS enables; calculation of the exact molecular mass of the sample components, identification of unknown compounds via molecular mass determination, quantification of known compounds determination of structure and chemical properties of molecules. Mass spectrometry-based proteomics allows quantitative system-wide analysis of the proteome, including post-translational modifications, protein-protein interactions and cellular localization. Quantification of the entire set of proteins expressed in a complex biological system, for example mammalian cells, is possible with a high sensitivity and a reasonable amount of time. With the availability of genomic information the massive capacity for peptide identification by mass spectroscopy is being used to annotate gene sequences and to find new protein-coding genes and splicing variants. In combination with new approaches to isolate specific post-translational modifications, MS based studies are revealing a much higher order of proteome complexity in which most proteins are modified by post translational modifications that crosstalk in intricate mechanisms to regulate protein function. Protein affinity strategies allow purification of candidate proteins and their interacting partners, which are subsequently identified by MS.

These studies describe dynamic and context-specific protein-protein interaction networks and protein complexes. The improvements in sensitivity, robustness and high-throughput of MS based proteomics now permits applications in the clinical field, including the possibility of discovering disease-related biomarkers and screening molecular targets of candidate drugs.

Oxidative stress in diabetic neuropathy

Diabetes mellitus

Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose. Type II diabetes mellitus typically has a late onset and occurs when the body becomes resistant to insulin or does not make enough insulin. Type I diabetes mellitus typically has an early onset and occurs when the pancreas produces little or no insulin, as a result of autoimmune destruction of the beta cells of the islets of langerhans. According to diabetes UK, approximately 2.5 million people have diabetes mellitus (DM) in the uk, 5-10% have type I DM, and 85-95% have type II DM. The world health organisation estimates that over 300 million people worldwide will be diagnosed with diabetes by the year 2030.

Insulin signaling

Insulin is a hormone released by the pancreatic beta cells in response to elevated levels of nutrients in the blood. Insulin triggers the uptake of glucose, fatty acids and amino acids into liver, adipose tissue and muscle and promotes the storage of these nutrients in the form of glycogen, lipids, and protein respectively. The insulin receptor, located on the surface of target cells such as liver, fat and muscle, is composed of two extracellular alpha subunits and two transmembrane beta subunits linked together by disulfide bonds. Binding of insulin to the alpha subunit induces a conformational change resulting in phosphorylation of a number of tyrosine residues present in the beta subunit. These residues are recognized by phosphotyrosine-binding (PTB) domains of adaptor proteins such as members of the insulin receptor substrate family (IRS). Receptor activation leads to the phosphorylation of tyrosine residues on the IRS protein, some of which are recognised by the src homology (SH) domain of the p85 regulatory subunit of PI3- kinase. The catalytic subunit of PI3-kinase p110, then phosphorylates phosphatidylinositol (4,5) biphosphatase (ptdins (4,5)p2) leading to formation of ptdins (3,4,5)p3. A key downstream effector of ptdins (3,4,5)p3 is AKT, which is recruited to the plasma membrane. Activation of AKT also requires the protein kinase 3-phosphoinositide dependent protein kinase 1 (PDK1), which phosphorylates AKT. Once active, AKT enters the cytoplasm where it leads to the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3). A major substrate of GSK3 is glycogen synthase, an enzyme that catalyzes the final step in glycogen synthesis. Phosphorylation of glycogen synthase by GSK3 inhibits glycogen synthesis, therefore the inactivation of GSK3 by AKT promotes glucose storage as glycogen. Insulin inhibits the production and release of glucose by the liver by blocking gluconeogenesis and glycogenesis. Insulin directly controls the activity of metabolic enzymes by phosphorylation and dephosphorylation, and also regulates the expression of genes encoding hepatic enzymes involved in gluconeogenesis. Insulin stimulates glucose uptake into cells by inducing translocation of the glucose transporter, GLUT4, from intracellular storage to the plasma membrane. PI3-kinase and AKT play a role in GLUT4 translocation. In addition, a PI3-kinase independent pathway recruits GLUT4 to the plasma membrane. In this pathway, insulin receptor activation leads to phosphorylation

the bones, PTH stimulates the release of calcium in an indirect process through osteoclasts which ultimately results in bone resorption. PTH directly stimulates osteoblasts which increases their expression of RANKL, a receptor activator for nuclear factor kappa-beta ligand, allowing for the differentiation of osteoblasts and osteoclasts. PTH also inhibits the secretion of osteoprotegerin, allowing for preferential differentiation into osteoclasts. Osteoprotegerin normally competitively binds with RANKL diminishing the ability to form osteoclasts. Osteoclasts possess the ability to remodel the bones by dissolution and degradation of hydroxyapatite and other organic material releasing calcium into the blood.

Circulating PTH targets the distal convoluted tubule and collecting ducts of the kidney directing increasing calcium reabsorption. PTH decreases phosphate reabsorption at the proximal convoluted tubule of the kidney. Phosphate ions in serum form salts with calcium that are insoluble, resulting in a decreased plasma calcium. The reduction of phosphate ions, therefore, results in more ionized calcium in the blood.

PTH stimulates the production of the enzyme 1-alpha-hydroxylase in the proximal convoluted tubule. 1-alpha-hydroxylase catalyses the synthesis of active vitamin D-1, 25-dihydroxycholecalciferol from the inactive form, 25-hydroxycholecalciferol. Active vitamin D plays a role in calcium reabsorption in the distal convoluted tubule via calbindin D, a cytosolic vitamin D allows the absorption of calcium through an active transcellular pathway and a passive paracellular pathway.

calcitonin, a hormone produced by the parafollicular cells of the thyroid, acts in opposition to PTH by inhibiting osteoclasts, stimulating osteoblasts and increasing excretion of calcium in the urine by the kidneys.

Secondary hyperparathyroidism

Secondary hyperparathyroidism is a medical condition of excessive secretion of PTH by the parathyroid glands in response to hypoglycemia, with resultant hyperplasia of these glands. Secondary hyperparathyroidism is primarily seen in patients with chronic kidney disease (CKD). CKD progression is associated with phosphate overload, high levels of fibroblast growth factor -23. Significant decrease in the expression of klotho, and a reduction of renal calcitriol production. Calcitriol deficiency influences parathyroid set point for calcium regulated PTH secretion and decreases the expression of vitamin D and calcium receptors. indirectly, calcitriol deficiency also stimulates PTH secretion due to a decrease in intestinal calcium absorption. Secondary hyperparathyroidism is treated by administering vitamin D and calcium supplements. The drug cinacalcet is a calcimimetic drug that binds to the calcium sensing receptors on cells of the parathyroid gland, inhibiting PTH secretion.

Bone preparation techniques

The choice of procedure for the preparation of bone specimens is influenced by the initial diagnosis, the urgency of the case, and the depth of investigation. Procedures include; paraffin or frozen sections after decalcification, ground sections, or resins. Bone specimens vary from needle biopsies to whole limbs. Biopsies investigating possible tumors or infection are decalcified in acid or with a chelating agent and processed routinely. Assessment of metabolic bone disease requires demonstration of the relationship between mineralized and

non-mineralized bone, and therefore requires undecalcified sections, for example resin or block staining of mineral before decalcification, for example Tripp and Mackay method. X-ray may provide information on; nature and extent of bone lesion, which blocks required for staining, the progress of decalcification, and whether foreign material is present such as metal. Acid decalcification effects haematoxylin and eosin staining resulting in a paler stain. Trichrome stains collagen, PAS stains osteoid, alizarin red stains calcium, phosphates and carbonate. The mineral component will stain with silver nitrate, for example Von Kossa, Tripp and Mackay. Fluorescent labelling may be used in metabolic investigation, and is based on the uptake in vivo of tetracycline. Two spaced doses are given, the drug is localised at active bone sites and therefore the space between the two fluorescent lines indicates deposition rate. The biopsy is processed into resin, and viewed unstained with a short wave ultraviolet light. Morphometry (quantitative analysis of a form, a concept that involves measurement of size and shape, with an eyepiece graticule or computer aided) is sometimes useful to detect minor osteoporosis (reduced bone mass) or hyperosteoidosis (excess osteoid) which may not be obvious subjectively. Morphometry can also be used to assess the effects of a treatment regimen, for example in hyperparathyroid bone disease.

Pathologic calcification

Pathologic calcification is the abnormal tissue deposition of calcium salts, together with smaller amounts of iron, magnesium, and other mineral salts. Pathologic calcification can occur in almost any tissue structure. Normal serum calcium levels are relatively high but precipitation in vivo does not generally occur due to calcification-inhibiting macromolecules, such as osteopontin. The first detectable mineral deposited in vivo is a poorly crystallised apatite which perfects with age. They then continue to grow by extracting more calcium and phosphate from solution, termed secondary nucleation. Also common only in pathology is primary nucleation, where crystals grow on a different surface, by epitaxy. Pathological calcification usually is initiated by the biologic membranes of mitochondria or matrix vesicles. Mitochondria frequently initiate intracellular calcification. Matrix vesicles, derived from the outer membrane of cells by budding or cell disruption, initiate extracellular calcification in calcific tendonitis, apatite-deposition osteoarthritis, atherosclerosis, cardiac valvular calcification, tympanosclerosis, and other calcific diseases. Matrix vesicles and mitochondria usually initiate calcification through the interaction of phosphatase enzymes with calcium-binding phospholipids, both of which are membrane bound. Hydroxyapatite (HA) crystals are formed first within the protective microenvironment of the membrane-enclosed microspace. Once formed and exposed to the extracellular fluid, HA crystals can serve as nuclei or templates, thus supporting progressive, autocatalytic mineral crystal proliferation. Calcification in live cells usually starts in the mitochondria. The cell membrane pumps calcium out of the cell, the mitochondrial membrane pumps calcium into the mitochondria. Damage to the cell membrane rapidly causes an imbalance resulting in calcified mitochondria and cell death. The calcified mitochondria are then available for secondary nucleation.

Dystrophic calcification can be defined as a calcification that occurs in degenerated or necrotic tissue. It is associated with multiple clinical conditions, such as collagen vascular diseases. It involves the deposition of calcium in soft tissues despite no generalized disturbance in the calcium or phosphorus metabolism, and this is often seen at sites of

death with the absence of typical apoptotic features such as cell shrinkage and nuclear condensation, instead chondrocytes further increase in cell volume.

Calcification of the growth plate

The plasma protein fetuin-A/alpha2-HS-glycoprotein is an inhibitor of excessive mineralisation in the growth plate cartilage matrix, as evidenced in knockout murine experiments. Calcification occurs in the extracellular matrix of the hypertrophic zone of the growth plate when the extracellular matrix volume is reduced to a minimum and alkaline phosphatase content is maximal. The concentrations within the extracellular matrix of the C-propeptide of type II collagen, aggregating proteoglycan, and hypertrophic zones, being maximal at the time of mineralisation. Total collagen concentration in the extracellular matrix initially increases through the proliferating and maturing zones but rapidly decreases in the hypertrophic zone. This is associated with the unwinding of the triple helix of type II collagen which starts in the pericellular sites in the zone of maturation, when type X collagen is first synthesized, and then extends throughout the hypertrophic zone.

Vascular calcification

Examples of non vascular calcification include valvular accumulation of calcium salts. Aortic valve calcification can sometimes be seen on chest radiography, and the presence of such calcifications is a sensitive marker for clinically significant aortic stenosis. The severity and location of aortic valve calcifications on chest CT are associated with an increased pressure gradient across the aortic valve. Examples of vascular calcification include: vascular mammary calcification. Vascular calcification can lead to the following conditions depending on where in the body calcification is occurring: atherosclerosis, diabetes, hypercholesterolemia, and chronic renal insufficiency. Vascular calcification is associated with an increased risk of; amputation due to damage to vasculature and consequent poor perfusion of tissues resulting in necrosis of cells thus necessitating amputation, ischaemic heart disease as a result of calcification occurring in blood vessels surrounding the heart, stroke, and mortality.

The link between tissue calcification and cardiovascular disease is termed endarteritis deformans (deformity of the arterial tunica intima). Vascular structure; tunica intima, the innermost layer composed of endothelial cells lining the vessel lumen; tunica media, the middle layer composed of smooth muscle cells and elastic fibres which allow for constriction of blood vessels to control blood flow; and tunica adventitia, the outermost layer composed of collagen fibres which maintain structural integrity of the vessel.

The majority of vascular calcification occurs in the tunica intima. Calcification can occur in the tunica media of peripheral arteries and is known as Monckeberg's sclerosis and occurs mainly in older, diabetic patients. Calcification of the tunica intima is seen in atherosclerosis. The initial step in calcification of the tunica intima is the accumulation of lipid rich macrophages and T-lymphocytes in the vessel wall forming fatty streaks, followed by invasion of vascular smooth muscle cells (VSMCs). VSMCs migrate from the tunica media to the tunica intima in response to various stimuli. VSMCs then proliferate and initiate synthesis of the extracellular matrix (ECM). The ECM is then calcified.