DEAR STUDENTS! 12-size letters are basic requirement, 10-size letters are extra requirement. Text in blue is facultative material.

TOOTH NUMBER ABNORMALITIES

5.- 7. HYPERDONTIA is the condition of having supernumerary teeth, or teeth which appear in addition to the regular number of teeth. The most common supernumerary tooth is a mesiodens, which is a malformed, peg-like tooth that occurs between the maxillary central incisors. Fourth and fifth molars that form behind the third molars are another kind of supernumerary teeth. Another rare type of supernumerary teeth is a "third set of teeth" that forms underneath and pushes out the second set of teeth, much like the second set formed underneath which pushes out the first set of teeth. The occurrence of the "third set of teeth" shows us that we can learn how to genetically engineer extra teeth. Many supernumerary teeth never erupt, but they may delay eruption of nearby teeth or cause other dental problems. Molar-type extra teeth are the rarest form. Hyperdontia is seen in a number of disorders, including Gardner's syndrome and cleidocranial dysostosis where multiple supernumerary teeth are seen that are usually impacted. It is suggested that supernumerary teeth develop from a third tooth bud arising from the dental lamina near the permanent tooth bud or possibly from splitting the permanent tooth bud itself. Supernumerary teeth in deciduous dentition are less common than in permanent dentition.

8.-10. GARDNER'S SYNDROME is a genetic disorder characterized by the presence of multiple polyps in the colon together with tumors outside the colon. The extracolonic tumors may include osteomas of the skull and thyroid cancer. The countless polyps in the colon predispose to the development of colon cancer. Polyps can also grow in the stomach, duodenum and small bowel. Inheritance: Gardner's syndrome is inherited in an autosomal dominant manner. Diagnosis. Gardner's syndrome can be identified based on oral findings, including multiple impacted and supernumerary teeth, multiple jaw osteomas which give a "cotton-wool" appearance to the jaws, as well as multiple odontomas, congenital hypertrophy of the retinal pigment epithelium (CHRPE), in addition to multiple adenomatous polyps of the colon. Genetics. Gardner's syndrome is now known to be caused by mutation in the APC gene (see later in Wnt signaling) located in chromosome 5q21 (band q21 on chromosome 5). This is the same gene as is mutant in familial adenomatous polyposis (FAP), a more common disease that also predisposes to colon cancer. After existing for most of the second half of the 20th century, Gardner's syndrome has vanished as a separate entity. It has been merged into familial adenomatous polyposis (FAP) and is now considered simply a phenotypic variant of FAP.

11.-14. CLEIDOCRANIAL DYSPLASIA Cleidocranial dysplasia is a rare condition (1 per million individuals) that primarily affects the development of the bones and teeth. Signs and symptoms of cleidocranial dysplasia can vary widely in severity, even within the same family. Individuals with cleidocranial dysplasia usually have underdeveloped or absent collarbones (clavicles). As a result, their shoulders are narrow and sloping, can be brought unusually close together in front of the body, and in some cases the shoulders can be made to meet in the middle of the body. Delayed closing of the spaces between the bones of the skull (fontanels) is also characteristic of this condition. The fontanels usually close in early childhood, but may remain open into adulthood in people with this disorder. Affected individuals may be 3 to 6 inches shorter than other members of their family, and may have short, tapered fingers and broad thumbs; short forearms; flat feet; knock knees; and an abnormal curvature of the spine (scoliosis). Characteristic facial features may include a wide, short skull (brachycephaly); a prominent forehead; wide-set eyes (hypertelorism); a flat nose; and a small upper jaw. Individuals with cleidocranial dysplasia may have decreased bone density (osteopenia) and may develop osteoporosis, a condition that makes bones progressively more brittle and prone to fracture, at a relatively early age. Women with cleidocranial dysplasia have an increased risk of requiring a cesarean section when delivering a baby, due to a narrow pelvis preventing passage of the infant's head. Dental abnormalities seen in cleidocranial dysplasia may include delayed loss of the primary (baby) teeth; delayed appearance of the secondary (adult) teeth; unusually shaped, peg-like teeth; malalignment of the teeth and jaws (malocclusion); and extra teeth, sometimes accompanied by cysts in the gums. In addition to skeletal and dental abnormalities, people with cleidocranial dysplasia may have hearing loss and be prone to sinus and ear infections. Some young children with this condition are mildly delayed in the development of motor skills such as crawling and walking, but intelligence is unaffected. Missense, nonsense point mutations and deletions in the RUNX2 gene cause cleidocranial dysplasia. The RUNX2 gene provides instructions for making a protein that is involved in bone and cartilage development and maintenance. This protein is a transcription factor. Researchers believe that the RUNX2 protein acts as a "master switch," regulating a number of other genes involved in the development of cells that build bones (osteoblasts). The mutations reduce or eliminate the activity of the protein produced from one copy of the RUNX2 gene in each cell, decreasing the total amount of functional RUNX2 protein. This shortage of functional RUNX2 protein interferes with normal bone and cartilage development, resulting in the signs and symptoms of cleidocranial dysplasia. This condition is inherited in an autosomal dominant pattern (haploinsufficiency), which means one copy of the normal gene is insufficient to do its job in
the cells. In some cases, an affected person inherits the mutation from one affected parent. Other cases may result from new mutations in the gene.

15. RUNX1 is essential for hematopoiesis and the malfunction of RUNX1 is responsible for 30% of human acute leukemias. RUNX1 is the most frequent target of chromosome translocations. RUNX3 is a major growth regulator of gastric epithelial cells and functions as a tumor suppressor of gastric cancer. In addition, RUNX3 was found to be a major target of TGF-β signaling cascade which is often considered as a tumor suppressor pathway.

16.-20. ANODONTIA
Anodontia, also called anodontia vera, is a rare genetic disorder characterized by the congenital absence of all primary or permanent teeth. It is associated with the group of skin and nerve syndromes called the ectodermal dysplasias. Anodontia is usually part of a syndrome and seldom occurs as an isolated entity. Partial anodontia, known as hypodontia or oligodontia, is the congenital absence of one or more teeth. Congenital absence of all wisdom teeth, or third molars, is relatively common. Treatment: replacement of missing teeth is possible using dental implant technology or denture.

21.-22. PAX9
This gene is a member of the paired box (PAX) family of transcription factors. PAX9 is responsible for tooth development and may more generally involve development of stratified squamous epithelia as well as various organs and skeletal elements. PAX9 plays a role in the absence of wisdom teeth in some human populations.

24.-25. INCONTINENTIA PIGMENTI
The skin lesions evolve through characteristic stages: blistering (from birth to about four months of age), a wart-like rash (for several months), swirling macular hyperpigmentation (from about six months of age into adulthood), followed by linear hypopigmentation. Alopecia, hypodontia, abnormal tooth shape, and dystrophic nails are observed. Some patients have retinal vascular abnormalities predisposing to retinal detachment in early childhood. Cognitive delays/mental retardation are occasionally seen. Discolored skin is caused by excessive deposits of melanin (normal skin pigment). Most newborns with IP will develop discolored skin within the first two weeks. The pigmentation involves the trunk and extremities, is slate-grey, blue or brown, and is distributed in irregular marbled or wavy lines. The discoloration sometimes fades with age. Neurological problems can include: cerebral atrophy, the formation of small cavities in the central white matter of the brain, and the loss of neurons in the cerebellar cortex. About 20% of children with IP will have slow motor development, muscle weakness in one or both sides of the body, mental retardation, and seizures. They are also likely to have visual problems, which can include: crossed eyes, cataracts, and severe visual loss. Dental problems are common, and include missing or peg-shaped teeth - patients with IP often keep milk teeth into adult life. Skeletal and structural anomalies can occur in approximately 14% of patients. The diagnosis of IP is established by clinical findings and occasionally by corroborative skin biopsy. Molecular genetic testing of the NEMO IKBK gene (chromosomal locus Xq28) reveals disease-causing mutations in about 80% of probands. Such testing is available clinically. IP is inherited in an X-linked dominant manner. IP is lethal in most, but not all, males. A female with IP may have inherited the IKBK mutation from either parent or have a new gene mutation. Parents may either be clinically affected or have germline mosaicism. Affected women have a 50% risk of transmitting the mutant IKBK allele at conception; expected ratio for liveborn children is 33% unaffected females, 33% affected females, and 33% unaffected males. Genetic counseling, prenatal testing, and preimplantation genetic diagnosis is available. In females, the cells expressing the mutated IKBK gene due to lyonization selectively die around the time of birth so the X-inactivation is extremely skewed. IP is caused by mutations in a gene called NEMO (NF-kappaB essential modulator). NEMO encodes the regulatory subunit of the inhibitor of kappaB kinase (IKK) complex, which activates NF-kappaB resulting in activation of genes involved in inflammation, immunity, cell survival, and other pathways. Mutations in this gene result in incontinentia pigmenti, hypohidrotic ectodermal dysplasia, and several other types of immunodeficiencies. Since NEMO helps activate NF-kB, which protects cells against TNF-alpha induced apoptosis, a lack of NEMO (and hence a lack of active NF-kB) makes cells more prone to apoptosis.

26. Mechanism of NF-kB action. In this figure, the NF-kB heterodimer between Rel and p50 proteins is used as an example. While in an inactivated state, NF-kB is located in the cytosol complexed with the inhibitory protein IκBα. Through the intermediacy of integral membrane receptors, a variety of extracellular signals can activate the enzyme IκB kinase (IKK). IKK, in turn, phosphorylates the IκBα protein, which results in ubiquitination, dissociation of IκBα from NF-κB, and eventual degradation of IκBα by the proteosome. The activated NF-kB is then translocated into the nucleus where it binds to specific sequences of DNA called response elements (RE). The DNA/NF-κB complex then recruits other proteins such as coactivators and RNA polymerase, which transcribe downstream DNA into mRNA, which, in turn, is translated into protein, which results in a change of cell function.

27.-29. REIGER’S SYNDROME
Partial or complete anodontia (oligodontia). Most commonly missing primary and permanent maxillary incisors. Microodontia—typically small cone shaped anterior teeth. Relative mandibular prognathism due to hypoplastic maxilla
Autosomal Dominant Inheritance. Dental, ocular and other abnormalities. Mutation in Pitx2/Reig gene causes this condition Pituitary homeobox 2 is a protein that in humans is encoded by the PITX2 gene. This gene encodes a member of the RIEG/PITX homeobox family, which is in the bicoid class of homeodomain proteins. This protein acts as a transcription factor and regulates
procollagen lysyl hydroxylase gene expression. Mutations in this gene are associated with Rieger syndrome, iridogoniodygenesis syndrome (IGDS), and sporadic cases of Peters anomaly. This protein is involved in the development of the eye, tooth and abdominal organs.

30. WITKOP’S TOOTH AND NAIL SYNDROME
autosomal dominant, nail dysgenesis, missing and malformed teeth, a mutation in MSX1 gene

31. VAN DER WOUDE SYNDROME
Syndrome occurring in 2% of patients with facial clefts, autosomal dominant with variable expressivity.
Mutations in IRF6 (interferon regulatory factor) responsible for most cases. Syngnathia: Epithelial strands running from the maxilla to the mandible

32. PAPILLON-LEFEVRE SYNDROME
Autosomal Recessive, Skin lesions, Chromosome 11q14.1 – q14.3. Defective Gene: Cathepsin C

33.-38. ECTODERMAL DYSPLASIA
Hyphodontic ectodermal dysplasia belongs to a heterogeneous group of disorders of the ectodermal tissue. Over 120 different types of ectodermal dysplasia have been reported. They are characterized by a number of defects involving the teeth, skin, and appendageal structures (hair, nails, and eccrine and sebaceous glands). Generally, this syndrome is an X-linked recessive disorder caused by EDA1 gene within the region Xq12-q13.1 and characterized by a triad of defects including: Hypotrichiosis, Hypotrichosis and Anomalous dentition: small pointed conical incisors, absent teeth (hypodontia to anodontia). Hair: hypotrichosis, alopecia, fine, dry, silky, hypochromic hair. Craniofacial features: saddle-nose: low nasal bridge, small nose with hypoplastic alae nasi, frontal bossing, prominent supraorbital ridges, prominent lips due to reduced lower facial height, periorbital pigmentation, small palatal and cranial base widths. Ectodysplasin-A is a protein that in humans is encoded by the EDA gene. The protein encoded by this gene is a membrane protein that can be cleaved to produce a secreted form. The encoded protein, which belongs to the tumor necrosis factor family, acts as a homotrimer and may be involved in cell-cell signaling during the development of ectodermal organs. Specifically, it is critical for interactions between two embryonic cell layers called the ectoderm and the mesoderm. In the early embryo, these cell layers form the basis for many of the body's organs and tissues. Ectoderm-mesoderm interactions are essential for the formation of several structures that arise from the ectoderm, including the skin, hair, nails, teeth, and sweat glands. More than 80 different mutations in the EDA gene have been identified in people with hypodontic ectodermal dysplasia. These mutations cause the X-linked form of the disorder, which accounts for 95 percent of all cases of hypodontic ectodermal dysplasia.

40. DENTINOGENESIS IMPERFECTA
Dentinogenesis imperfecta (hereditary Opalescent Dentin) is a genetic disorder of tooth development. This condition causes teeth to be discolored (most often a blue-gray or yellow-brown color) and translucent. Teeth are also weaker than normal, making them prone to rapid wear, breakage, and loss. These problems can affect both primary (baby) teeth and permanent teeth. This condition is inherited in an autosomal dominant pattern. Dentinogenesis imperfecta affects an estimated 1 in 6,000 to 8,000 people. Researchers have described three types of dentinogenesis imperfecta with similar dental abnormalities. Type I: Occurs in people who have osteogenesis imperfecta, a genetic condition in which bones are brittle and easily broken. It is usually an autosomal dominant trait with variable expressivity but can be recessive if the associated osteogenesis imperfecta is of recessive type. Type I occurs as part of osteogenesis imperfecta, which is caused by mutations in Collagen (COL1A1 and COL1A2) genes. This type is no longer considered true dentinogenesis imperfecta as the mutations occur in different genes in the two conditions, though the clinical and radiological features are the same. Type II: Occurs in people without other inherited disorders ie Osteogenesis imperfecta. It is an autosomal dominant trait. A few families with type II have progressive hearing loss in addition to dental abnormalities. Type II and type III may be the same disorder. Mutations in the DSPP gene have been identified in people with type II and type III dentinogenesis imperfecta. The DSPP gene provides instructions for making three proteins that are essential for normal tooth development. These proteins are involved in the formation of dentin. Mutations in the DSPP gene may affect the proteins made by the gene, leading to the production of abnormally soft dentin. Teeth with defective dentin are discolored, weak, and more likely to decay and break. The DSPP gene provides instructions for making a protein called dentin sialophosphoprotein. Soon after it is produced, this protein is cut into two smaller proteins: dentin sialoprotein and dentin phosphoprotein. These proteins are components of dentin, which is a bone-like substance that makes up the protective middle layer of each tooth. DSPP-derived proteins are essential for normal tooth development. Dentin phosphoprotein is thought to be involved in the normal hardening of collagen, the most abundant protein in dentin. Specifically, dentin phosphoprotein may play a role in the deposition of mineral crystals among collagen fibers (mineralization). More than 20 mutations in the DSPP gene have been identified in people with dentinogenesis imperfecta. The teeth may be gray to yellowish brown. They exhibit translucent or opalescent hue. Enamel is usually lost early. The teeth however are not more susceptible to dental caries than normal ones. Radiographic features: Type 1 and 2 show total obliteration
of the pulp chamber. Type 3 shows thin dentin and extremely enormous pulp chamber. These teeth are usually known as Shell Teeth.

41. AMELOGENESIS IMPERFECTA
Genetics Up to date, mutations in the AMELX, ENAM, MMP20, and KLK-4 genes have been found to cause amelogenesis imperfecta (non-syndromic form). The AMELX, ENAM, KLK-4 and MMP20 genes provide instructions for making proteins that are essential for normal tooth development. These proteins are involved in the formation of enamel, which is a hard, calcium-rich material that forms the protective outer layer of each tooth. Mutations in any of these genes alter the structure of these proteins or prevent the genes from making any protein at all. As a result, tooth enamel is abnormally thin or soft and may have a yellow or brown color. Teeth with defective enamel are weak and easily damaged. Amelogenesis imperfecta can have different inheritance patterns depending on the gene that is altered. Most cases are caused by mutations in the ENAM gene and are inherited in an autosomal dominant pattern. Amelogenesis imperfecta is also inherited in an autosomal recessive pattern; this form of the disorder can result from mutations in the ENAM or MMP20 gene. About 5% of amelogenesis imperfecta cases are caused by mutations in the AMELX gene and are inherited in an X-linked pattern. Treatment: crowns are sometimes being used to compensate for the soft enamel. Usually stainless steel crowns are used in children which may be replaced by porcelain once they reach adulthood. In the worst case scenario, the teeth may have to be extracted and implants or dentures are required. Epidemiology: the exact incidence of amelogenesis imperfecta is uncertain. Estimates vary widely, from 1 in 700 people in northern Sweden to 1 in 14,000 people in the United States.

42.-44. EHLERS-DANLOS SYNDROME
symptoms: hyperelasticity of skin, skin fragility, joint laxity, ligamentous shortening, Aortic Aneurysms, Ocular fragility
dental findings: hypoplastic enamel, large pulp stones, malformed, stunted roots
mutations of several collagen genes lead to this syndrome.
inheritance: autosomal dominant

48.-52. BASICS OF HUMAN EMBRYOGENESIS
A germ layer, occasionally referred to as a germinal epithelium, is a group of cells, formed during animal embryogenesis. Germ layers are particularly pronounced in the vertebrates; however, all animals more complex than sponges produce two or three primary tissue layers (sometimes called primary germ layers). Animals with radial symmetry, like cnidarians, produce two germ layers (the ectoderm and endoderm) making them diploblastic. Animals with bilateral symmetry produce a third layer between these two layers (appropriately called the mesoderm) making them triploblastic. Germ layers eventually give rise to all of an animal’s tissues and organs through the process of organogenesis. Fertilization leads to the formation of a zygote. During the next stage, cleavage, mitotic cell divisions transform the zygote into a tiny ball of cells, a blastula. This early embryonic form undergoes gastrulation, forming a gastrula with either two or three layers: ectoderm, mesoderm, endoderm. In all vertebrates, these are the forerunners of all adult tissues and organs. The endoderm forms: the stomach, the colon, the liver, the pancreas, the urinary bladder, the lining of the urethra, the epithelial parts of trachea, the lungs, the pharynx, the thyroid, the parathyroid, and the intestines. The mesoderm forms: skeletal muscle, the skeleton, the dermis of skin, connective tissue, the urogenital system, the heart, blood (lymph cells), and the spleen. The ectoderm forms: the central nervous system, the lens of the eye, cranial and sensory, the ganglia and nerves, pigment cells, head connective tissues, the epidermis, hair, and mammary glands. Because of its great importance, the so called neural crest is sometimes considered a fourth germ layer. It is, however, derived from the ectoderm. Neural crest cells are a transient, multipotent, migratory cell population unique to vertebrates that gives rise to a diverse cell lineage including melanocytes, craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons, glia and the odontoblasts of the tooth primordia.

52. After gastrulation, neural crest cells are specified at the border of the neural plate and the non-neural ectoderm. During neuration, the borders of the neural plate, also known as the neural folds, converge at the dorsal midline to form the neural tube. Subsequently, neural crest cells from the roof plate of the neural tube undergo an epithelial to mesenchymal transition, delaminating from the neuroepithelium and migrating through the periphery where they differentiate into varied cell types. A molecular cascade of events is involved in establishing the migratory and multipotent characteristics of neural crest cells. First, extracellular signaling molecules, secreted from the adjacent epidermis and underlying mesoderm such Wnts, BMPs and Fgfs separate the non-neural ectoderm (epidermis) from the neural plate during neural induction. Wnt signaling has been demonstrated in neural crest induction in several species. In coherence with this observation, the promoter region of slug (a neural crest specific gene) contains a binding site for transcription factors involved in the activation of Wnt-dependent target
genes, suggestive of a direct role of Wnt signaling in neural crest specification. The current role of BMP in neural crest formation is associated with the induction of the neural plate. BMP antagonists diffusing from the ectoderm generates a gradient of BMP activity. In this manner, the neural crest lineage forms from intermediate levels of BMP signaling required for the development of the neural plate (low BMP) and epidermis (high BMP). Fgf from the paraxial mesoderm has been suggested as a source of neural crest inductive signal. Our current understanding of the role of BMP, Wnt, and Fgf pathways on neural crest specifier expression remains incomplete. Cranial neural crest migrates dorsolaterally to form the craniofacial mesenchyme that differentiates into various cranial ganglia and craniofacial cartilages and bones. These cells enter the pharyngeal pouches and arches where they contribute to the thymus, bones of the middle ear and jaw and the odontoblasts of the tooth primordial.

53.-55. TOOTH DEVELOPMENT

Teeth are organs that are only found in the oral cavity of vertebrates. Although they are composed of mineralized tissues and they are attached to bone, they do not form as outgrowths of bone. In fact, tooth development starts in the embryonic oral epithelium well before bone formation, and osteogenesis of the alveolar bone is later regulated by the teeth rather than vice versa. The mineralizing extracellular matrices of teeth, the enamel, dentine, and cementum, are formed by the ameloblasts, odontoblasts, and cementoblasts, respectively, which are unique dental cell types differentiating during specific stages of tooth morphogenesis. Teeth are typical examples of epithelial appendages, i.e., organs that develop from surface epithelium and underlying mesenchymal tissue. Interactions between the epithelial and mesenchymal tissues regulate the development of all epithelial appendages. During tooth development four signaling pathways (hedgehog, fibroblast growth factor, bone morphogenetic protein and wnt) operate. The hedgehog and fibroblast growth factor pathways instruct the cells to proliferate, while the bone morphogenetic protein and wnt pathways regulate differentiation of the cells determining their identity. FGF-8 and BMP-4 are early signals in the oral ectoderm, which act on the underlying neural crest-derived mesenchyme and induce the capacity to form teeth, and determine tooth identity, respectively. The oral ectoderm subsequently thickens and starts to bud, and several signal molecules belonging to all four signal families (BMP, FGF, Wnt, Shh) are expressed in the epithelium. The dental mesenchyme condenses around the invaginating epithelium and expresses signals that reciprocally act on the epithelium, which then folds and develops to the cap and bell stages. A signaling center, the enamel knot, that appears at the tip of the epithelial bud as it transforms from the bud to cap stage. In this transient epithelial structure, several signal molecules are coexpressed, and it is suggested that the enamel knot is involved in the regulation of tooth shape.

56.-60. HEDGEHOG SIGNALING

The evolutionarily conserved Hedgehog pathway plays a critical role in a time and position-dependent fashion during development by regulating patterning and maintenance of proliferative niches. In the absence of ligand, the Hh signalling pathway is inactive. In this case, the transmembrane protein receptor Patched (Ptc) inhibits the activity of Smoothened (Smo), a seven transmembrane protein. The transcription factor Gli, a downstream component of Hh signalling, is prevented from entering the nucleus through interactions with cytoplasmic proteins, including Fused and Suppressor of fused (Sufu). As a result, transcriptional activation of Hh target genes is repressed. Activation of the pathway is initiated through binding of any of the three mammalian ligands — Sonic hedgehog, Desert hedgehog or Indian hedgehog (all are represented as Hh in the figure) — to Ptc. Ligand binding results in de-repression of Smo, thereby activating a cascade that leads to the translocation of the active form of the transcription factor Gli to the nucleus. Nuclear Gli activates target gene expression, including Ptc and Gli itself, as well as Hip, a Hh binding protein that attenuates ligand diffusion. Other target genes that are important for the oncogenic function of the Hh pathway are genes that are involved in controlling cell proliferation (cyclin D, cyclin E, Myc and components of the epidermal-growth-factor pathway) and in angiogenesis (components of the platelet-derived-growth-factor and vascular-epithelial-growth-factor pathway).

59. HEDGEHOG SIGNALING AND CANCER

Misregulation of Hedgehog (Hh) signalling causes cancer in different tissues. Ptc mutations that are associated with basal-cell carcinoma (BCC), as well as with medulloblastoma and rhabdomyosarcoma. Cancer associated mutations are usually loss-of-function alleles, so Ptc can be considered to be a tumour suppressor. Similarly, loss-of-function mutations in Suppressor of fused (Sufu) have also been identified in some medulloblastoma cells. Constitutively active forms of Smo are oncogenic and can function independently of ligand binding to Ptc, leading to BCC. An oncogenic form of Shh has been associated with BCC, whereas ectopic expression of Gli has been shown to cause glioma. Gli is inhibited by protein kinase A (Pka). Misregulation of Hh signalling has also been associated with pancreatic adenocarcinoma, oesophageal and stomach cancer, and small-cell lung cancer. Little is known about the molecular mechanisms by which Hh signalling is upregulated in these tumours.

60. HEDGEHOG SIGNALING IN DETAIL

Proper secretion and gradient diffusion of the vertebrate hedgehog-family ligands, including Sonic, Desert, and Indian hedgehog all require autoprocessive cleavage and cholesterol as well as palmitate lipid modifications. In the absence of hedgehog ligand in the receiving cell (Off-state), the receptor for hedgehog-family ligands, patched, is normally bound to and represses the activity
of another transmembrane protein called smoothened, possibly by preventing its membrane association. In the Off-state, SUFU and COS2 sequester the microtubule-bound pool of the transcription factor Gli in the primary cilium. Gli can be phosphorylated by PKA, CK1, and GSK3 resulting in β-TrCP-mediated degradation of Gli activators (Gli1 and Gli2 in mammals) or in the conserved pathway generation of repressor-Gli (Gli3 or truncated-Ci in Drosophila), which leads to repression of hedgehog target genes. In the On-state, hedgehog binding to patched leads to activation and translocation of smoothened to the primary cilium. Its associated G-protein activity likely blocks inhibitory kinase action on Gli which is now free to translocate to the nucleus and activate hedgehog target genes, including cyclin D, cyclin E, MYC, and patched. Consequently, the conserved action of hedgehog ligands is to switch the Gli-factors from being transcriptional repressors to activators.

61-66. MAPK PATHWAY/FGF SIGNALING
The main role of the MAPK/ERK pathway (mitogen-activated protein kinase) is to induce cell division in a cell. More precisely, a cell in G1 or in G0 is instructed to start the cell cycle. In a multicellular organism, cells do not divide whenever they want, only if it is needed for the whole organism (growing or regeneration). Cells secrete proteins called growth factors which bind to their transmembrane growth factor receptors transmitting the signal to the cytoplasm. Through a series of proteins eventually transcription factors will be activated, and the gene expression pattern of the target cells changes. The newly expressed genes play a role in cell cycle progression and cell division. The growth factors and their receptors are cell type specific (fibroblast growth factor, nerve growth factor, platelet derived growth factor). The growth factor receptors are dimeric transmembrane proteins. When the growth factor binds from the extracellular side, tyrosin kinase domains get activated at the cytoplasmic side of the receptor. The two receptor cross phosphorylate each other’s cytoplasmic tyrosin residues creating attachment sites for adaptor molecules. The adaptors (Shc, GRB2, Crk, etc.) linking the receptor to a guanine nucleotide exchange factor (SOS, C3G, etc.) transducing the signal to small GTP binding proteins (Ras, Rap1), which in turn activate the core unit of the cascade composed of a MAPKKK (Raf), a MAPKK (MEK1/2), and MAPK (Erk). An activated Erk dimer can regulate targets in the cytosol and also translocate to the nucleus where it phosphorylates a variety of transcription factors (blue on slide 62.) regulating gene expression. All components of the MAPK pathway are considered as protooncogenes. Protooncogenes are normal genes which stimulate cell cycle progression and cell division. Their dominant, gain of function mutations (resulting in constitutively active proteins) turns them to oncogenes. Oncogenes lead to uncontrolled cell division, hallmark of all cancers.

63. The MAPK/Erk signaling cascade is activated by a wide variety of receptors involved in growth and differentiation including the aforementioned receptor tyrosine kinases (RTKs), integrins, and ion channels. The specific components of the cascade vary greatly among different stimuli.

64. The FGF signaling not only activates the MAPK pathway (left) but the phosphorylated FGFR activates phospholipase C (right), resulting in IP3 and DAG second messengers, intracellular Ca++ concentration increase and protein kinase C activation.

65. The image shows a transgenic mouse embryo on its 14th day of embryonic development. The embryo carries a ‘lacZ reporter’ gene that reports the activity of a key member of the Fibroblast Growth Factor (FGF) family of signaling molecules. Treatment with X-gal has revealed the distribution of lacZ, and hence FGF, to show that FGF has a role in the outgrowth of digits during limb development.

66. The MAPK/Erk signaling cascade might result not only in cell division, but in certain cases cell differentiation, cell death, inflammation.

67.-69. BMP (bone morphogenetic protein) SIGNALING
Originally discovered by their ability to induce the formation of bone and cartilage, BMPs are now considered to constitute a group of pivotal morphogenetic signals, orchestrating tissue architecture throughout the body. The signaling pathways involving BMPs, BMPRs and Smads are important in the development of the heart, central nervous system, and cartilage, as well as post-natal bone development. BMPs belong to the Transforming growth factor beta superfamily of proteins. Transforming growth factor-β (TGF-β) superfamily signaling plays a critical role in the regulation of cell growth, differentiation, and development in a wide range of biological systems. In general, signaling is initiated with ligand-induced oligomerization of serine/threonine receptor kinases and phosphorylation of the cytoplasmic signaling molecules Smad2 and Smad3 for the TGF-β/activin pathway, or Smad1/5/8 for the bone morphogenetic protein (BMP) pathway. Carboxy-terminal phosphorylation of Smads by activated receptors results in their partnering with the common signaling transducer Smad4, and translocation to the nucleus. Activated Smads regulate diverse biological effects by partnering with transcription factors resulting in cell-state specific modulation of transcription.

68. The interplay between BMP and TGF-β signals determines germ layer/tissue identity.
The activin and BMP pathways are themselves attenuated by MAPK signaling at a number of levels, while the expression of inhibitory Smads (1-Smads) 6 and 7 is induced by both activin/TGF-β and BMP signaling as part of a negative feedback loop. In certain contexts, TGF-β signaling can also affect Smad-independent pathways, including Erk, SAPK/JNK, and p38 MAPK pathways. Activation of Smad-independent pathways through TGF-β signaling is also common. Rho GTPase (RhoA) activates downstream target proteins, such as mDia and ROCK, to prompt rearrangement of the cytoskeletal elements associated with cell spreading, cell growth regulation, and cytokinesis. Cdc42/Rac regulates cell adhesion through downstream effector kinases PAK, PKC, and c-Abl following TGF-β activation.

70.-73. WNT SIGNALLING
The name Wnt was coined as a combination of Wg (wingless) and Int and can be pronounced as ‘wint’. The wingless gene had originally been identified as a recessive mutation affecting wing and haltere development in Drosophila melanogaster. It was subsequently characterized as a segment polarity gene in Drosophila melanogaster that functions during embryogenesis and also during adult limb formation during metamorphosis. The INT genes were originally identified as vertebrate genes near several integration sites of mouse mammary tumor virus (MMTV). The Int-1 gene and the wingless gene were found to be homologous, with a common evolutionary origin evidenced by similar amino acid sequences of their encoded proteins. Mutations of the wingless gene in the fruit fly were found in wingless flies, while tumors caused by MMTV were found to have copies of the virus integrated into the genome forcing overproduction of one of several Wnt genes.

Wnts are a major class of secreted morphogenic ligands of profound importance in establishing the pattern of development in the bodies of all multicellular organisms studied. The Wnt/β-Catenin pathway regulates cell fate decisions during development. The Wnt-ligand is a secreted glycoprotein that binds to Frizzled receptors, which triggers a cascade resulting in gene expression change. During development, the Wnt/β-catenin pathway integrates signals from many other pathways including Retinoic acid, FGF, TGF-β, and BMP in many different cell-types and tissues.

71. APC (adenomatosis polyposis coli) is a human gene that is classified as a tumor suppressor gene. Tumor suppressor genes prevent the uncontrolled growth of cells that may result in cancerous tumors. The protein made by the APC gene plays a critical role in several cellular processes that determine whether a cell may develop into a tumor. The APC protein helps control how often a cell divides, how it attaches to other cells within a tissue, or whether a cell moves within or away from a tissue. This protein also helps ensure that the chromosome number in cells produced through cell division is correct. The APC protein accomplishes these tasks mainly through association with other proteins, especially those that are involved in cell attachment and signaling. The activity of one protein in particular, beta-catenin, is controlled by the APC protein. Regulation of beta-catenin prevents genes that stimulate cell division from being turned on too often and prevents cell overgrowth.

72. The (Adenomatosis Polyposis Coli) APC protein normally builds a complex with glycosynthase kinase 3beta(GSK 3β) and Axin. This complex is then able to bind β-catenins in the cytoplasm, that have dissociated from adherens junctions between cells. With the help of Casein Kinase 1 (CK1) which does the first phosphorylation of β-catenin, there is subsequent phosphorylation by GSK-3β. This targets β-catenin for ubiquitination and degradation by cellular proteosomes. This prevents it from translocating into the nucleus, where it acts as a transcription factor for proliferation genes. The deactivation of the APC protein can take place after certain chain reactions in the cytoplasm are started, e.g. through the Wnt signals that destroy the conformation of the complex. Mutations in APC often occur early on in cancers such as colon cancer. Patients with familial adenomatous polyposis (FAP=Gardner’s syndrome!) have germline mutations, with 95% being nonsense/frameshift mutations leading to premature stop codons. 33% of mutations occur between amino acids 1061-1309. In somatic mutations, over 60% occur within a mutation cluster region (1286-1513), causing loss of axin binding sites in all but 1 of the 20AA repeats. Mutations in APC lead to loss of β-catenin regulation, altered cell migration and chromosome instability.

73. The Wnt/β-Catenin pathway regulates cell fate decisions during development of vertebrates and invertebrates. The Wnt-ligand is a secreted glycoprotein that binds to Frizzled receptors, which triggers a cascade resulting in displacement of the multifunctional kinase GSK-3β from the APC/Axin/GSK-3β-complex. In the absence of Wnt-signal (Off-state), β-catenin, an integral cell-cell adhesion adaptor protein as well as transcriptional co-regulator, is targeted for degradation by the APC/Axin/GSK-3β-complex. Appropriate phosphorylation of β-catenin by coordinated action of CK1 and GSK-3β leads to its ubiquitination and proteosomal degradation through the β-TrCP/SKP complex. In the presence of Wnt binding (On-state), Dishevelled (Dvl) is activated by phosphorylation and poly-ubiquitination, which in turn recruits GSK-3β away from the degradation complex. This allows for stabilization of β-catenin levels, Rac1-dependent nuclear translocation and recruitment to the LEF/TCF DNA-binding factors where it acts as an activator for transcription by displacement of Groucho- HDAC corepressors. Additionally, in complex with the homeodomain factor Prop1, β-catenin has also been shown to act in context-dependent activation as well as repression complexes. Importantly, point-mutations in β-catenin lead to its deregulated stabilization. APC and Axin mutations have also been documented in some tumors, underscoring the deregulation of this pathway in human cancer. During development, the Wnt/β-catenin pathway integrates signals from many other pathways including Retinoic acid, FGF, TGF-β, and BMP in many different cell-types and tissues. In addition, GSK-3β is also involved in glycogen metabolism and other key pathways, which has made its inhibition relevant to diabetes and neurodegenerative disorders.