Embryological Basis of Congenital Tumours

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Introduction

During normal cellular development, there is a complex system of checks and balances to ensure regulation of the cells as they proliferate and specialize to perform their physiological functions. Various genes cooperate, concomitantly and/or sequentially with others, to activate and direct the developmental mechanisms in a developing foetus. After their target is achieved, these pathways are either kept dormant or are used elsewhere in a different context (growth, repair, etc.). Reactivation of these genes, by various mutations and/or carcinogens, can reinstate these developmental pathways. If these pathways remain active incessantly and do not obey the normal regulatory mechanisms, cell proliferation becomes independent of the growth stimulus, and this produces a mass—cancer [1–3].

Embryological Basis of Cancer

The growth and development of an embryo and that of a tumour has many parallels. Growth and development of a foetus from a single fertilized cell, the zygote, is a remarkable feat. This possibly is the best example of fast, coordinated growth where multiple complex pathways—like proliferation, migration, differentiation, apoptosis and reorganization—take place in an orchestrated manner. It is interesting to note that the growth of the embryo is faster than any of the malignant tumours [1–3].

As we continue to understand the developmental pathways operative in embryological development, startling revelations indicate that patterns of molecular pathways underpinning the tumour growth, migration, proliferation and differentiation have similar blueprints as the developmental pathways, which have either gone awry or have been arrested at a particular stage of development [2, 3]. This also sheds light of the timing of these tumours in relation to development.

Let us look at examples of the some of the many developmental/physiological pathways that are frequently deployed by tumour cells.

- The neural crest cells migrate to various locations in the body and are crucial in the development of many structures including the aortic arch, craniofacial cartilage and enteric nervous system [4]. This requires a very complex and intricate interplay of various developmental pathways. The tumour cells can use them selectively to metastasize to different locations in the body.
- Proliferation, migration and differentiation are vital in wound healing [1–3]. To enable repair of a skin wound, the epithelial cells of the skin must first transform into a mesenchymal phenotype. The epithelial cells are anchored to the basement membranes and other epithelial structures by desmosomes and hemi-desmosomes, respectively. To enable migration, they must lose their anchors and express laminins on their surfaces which help them to dissociate from their confines, interact with the extracellular connective tissue stroma and migrate to the wound area (epithelial to mesenchymal transition) [2, 3]. Angiogenesis supports this process. Once the skin wound has repaired, the mesenchymal phenotype reverts back to the epithelial type, and status quo is maintained. It is easy to see how these fundamental physiological pathways can be exploited by the various tumour cells to invade, migrate and metastasize.
- Formation of the placental bed during normal pregnancy is another fine example of many intricate pathways working in tandem. The aim of the exercise is to secure a constant and uniform blood supply from the host. The intermediate trophoblasts (X-cells) of the placenta invade deep into the maternal uterine wall to anchor the placenta and plug and remodel the uterine spiral arteriole vessels to

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establish the placental vascular bed. All of this happens whilst the mother's immune system is temporarily suppressed lest it should reject the foetus, which harbours paternal (foreign) antigens. Many tumours adopt these developmental strategies during tissue invasion, vascular invasion and evasion of immune surveillance [2, 3].

In essence, the tumour does not need to reinvent mechanisms to grow and disseminate throughout the body. Deregulation of physiological mechanisms may serve as important primary driver for cancer. Thus, the apparently random mutations that cancer cells may accrue may not just be chance events after all, and it may be plausible that the cancer cells selectively run through the chronicles of developmental works to serve its ends [2, 3].

The Cancer Model

Cancer is a genetic disease where disordered autonomous cell proliferation is driven by a series of accumulating genetic changes influenced by hereditary factors and the somatic environment. Apparently random mutations in the DNA of the cells enable them to escape from the clutches of stringent and tightly regulated developmental pathways [1–3, 5]. As a result, cancer cells do not remain a single disease, and as additional mutations accumulate, the cells within the mass become heterogeneous, and different tumour subclones appear, each diversifying and acquiring different characteristics to give the tumour a survival advantage. This randomness may also explain why different people with cancers arising from the same cells may be different in terms of their biological progression, prognosis and response to therapy [1–3, 5].

The dysregulated growth that characterizes neoplastic cells is caused by overexpression of proto-oncogenes (which move the cell through the cell cycle) or inactivation of tumour suppressor genes (which normally restrict cell growth and proliferation). These deranged genes may occur when the DNA has point mutations, viral insertions, chromosomal or gene amplifications, deletions or rearrangements which occur through genomic instability during cell divisions and hence are more likely to be accumulated with advancing age [5]. Certain tumours, specifically retinoblastoma and Wilms' tumour, occur earlier (and more often bilaterally) when they result from germline mutations (which have been inherited) than when they result from sporadic or somatic mutations. These observations led Knudson and Strong to propose a 'two-hit' mechanism of carcinogenesis in which the first genetic defect, already present in the germ line, must be complemented by an additional spontaneous mutation before a tumour can arise. In sporadic cancer, cellular transformation only occurs when two (or more) spontaneous mutations take place in the same cell [5].

As such, most tumours represent the disordered cell division associated with the genetic degeneration of age, as evidenced by statistics indicating that a third of adults in the UK will develop a tumour at some stage in their lives. Increasingly, the genetic basis of many tumours is being discovered including some with an inherited genetic predisposition (such as breast cancer [e.g. BRCA1] or colon cancer [e.g. APC]). Less commonly, tumours present in infancy or more rarely perinatally, and these 'congenital' tumours will be outlined in this chapter.

Nephroblastoma (Wilms' Tumour)

Wilms' tumour or nephroblastoma is a common kidney tumour of childhood with a peak age at onset of 3 years [5, 6] (Fig. 49.1). Development of Wilms' tumour appears to be intricately linked to the process of foetal nephrogenesis [7]. A genetic basis has long been postulated and remains the focus of much research [8]. In a kidney with Wilms' tumour, often there are associated adjacent foci of primitive renal blastemal tissue called *nephrogenic rests* (nephroblastomatosis). These are particularly common in children with an inherited susceptibility to Wilms' tumour and may represent a premalignant lesion. Wilms' tumour genes may therefore be involved in distinct developmental pathways in the kidney, and their inactivation may interrupt normal development resulting in increased risk of malignant transformation. Various genes have been implicated in this malignant transformation including two genes on the short arm of chromosome 11, the Wilms' tumour suppressor genes—WT1 (11p13) and WT2 (11p15) [5, 8].

Embryological basis of Wilms' tumour: The kidney develops from a structure called metanephros which itself is derived from two separate mesodermal components—the ureteric bud and the metanephric mesenchyme [5]. The ureteric bud invaginates into the metanephric mesenchyme, and as this happens, both reciprocally induce sequential activation of genes that activates signalling cascades which in turn stimulates successive dichotomous branching of the ureteric bud. This complex process involves mesenchyme-to-epithelial transition (MET) and requires cooperation of diverse players such as WT-1/WT-2, Wnt1/Wnt4, c-ret receptor tyrosine kinase, BMP4, glial-derived neurotrophic factor (GDNF) and the GDNF receptor (GDNFRα) [8] (Fig. 49.2). The tips of the distal branches then sequentially transform through the comma- and S-shaped body stage until vascular endothelial cells migrate into these bodies to form glomerular tufts; the remaining distal bodies elongate to form the renal collecting duct system, together forming the functional nephron. The subcapsular zone of the foetal kidney contains a region of undifferentiated mesenchyme (nephrogenic zone) which keeps adding rows of glomeruli to the kidney. Nephrogenesis is completed by 36 gestational weeks [8].

Classical Wilms' tumours show a typical triphasic histology—renal blastemal, epithelial components and stroma.

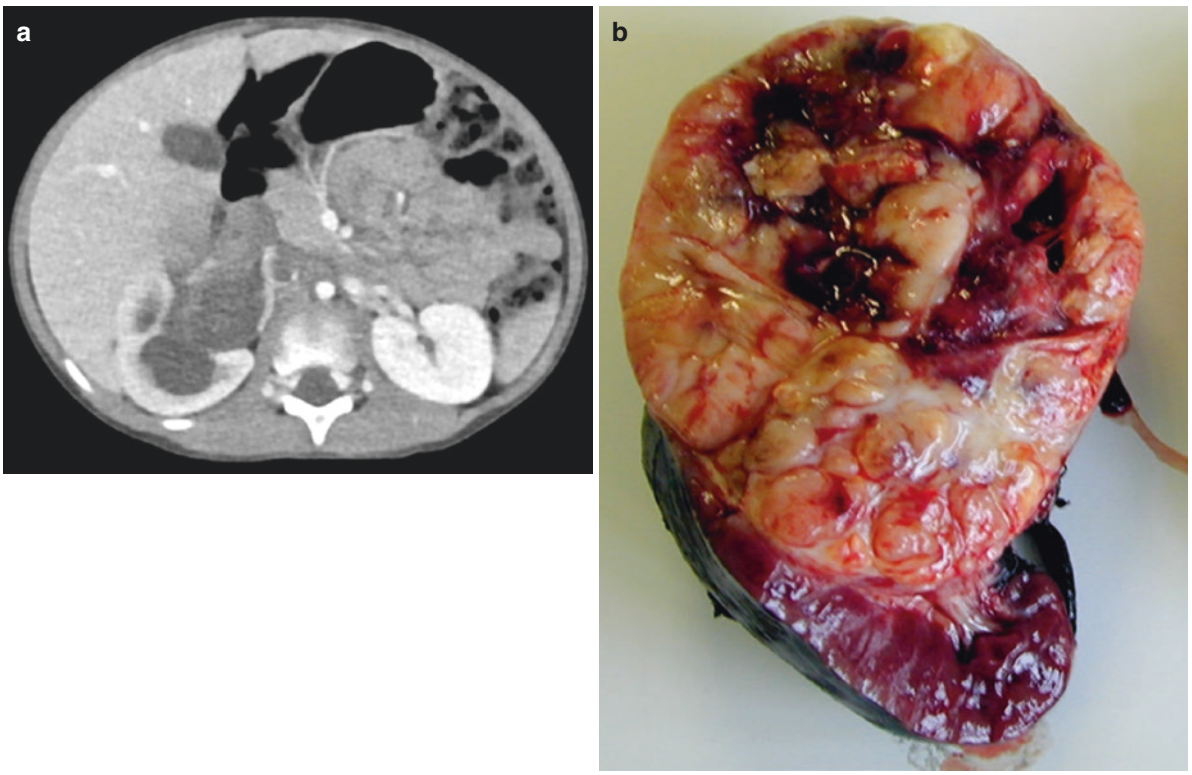

Fig. 49.1 Radiological and gross findings in Wilms' tumour. (**a**) MRI of the abdomen showing a large renal mass arising from the upper pole of the kidney. (**b**) Macroscopic appearance of Wilms' tumour arising from the upper pole of the kidney

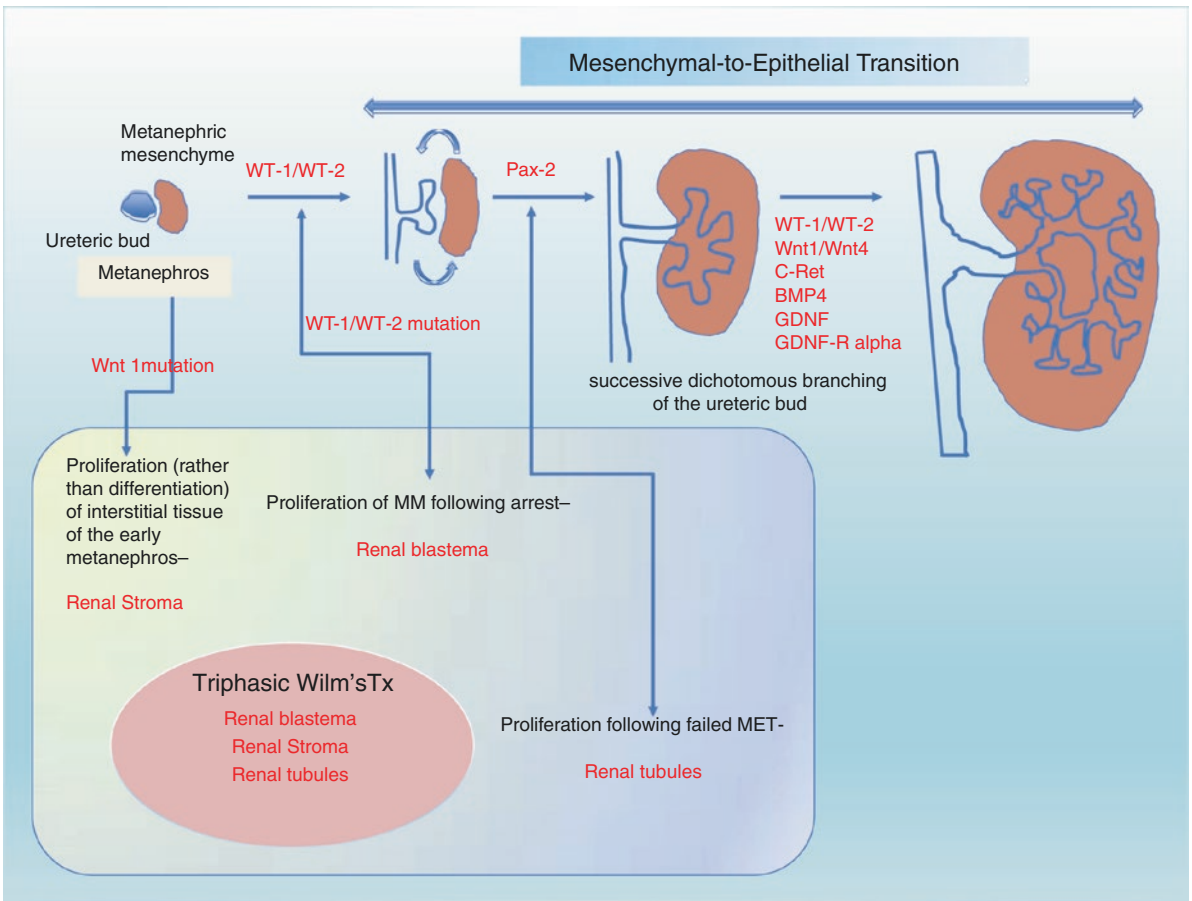

Fig. 49.2 Schematic diagram to show the development of Wilms' tumour

Each component of the tumour may reflect an arrested developmental stage of renal nephrogenesis [7–9].

Blastemal elements	• Proliferation (rather than differentiation) of undifferentiated metanephric mesenchyme • Driven by WT-1/WT-2
Epithelial elements	• Proliferation following partial differentiation of blastemal elements into renal tubules due to unsuccessful attempt at mesenchyme-to-epithelial transition (MET) • Driven by Pax-2
Stromal elements	• Proliferation (rather than differentiation) of interstitial tissue of the early metanephros • Driven by Wnt1

The histology of the Wilms' tumour shows varying proportions of these three components. Any of these elements may be the dominant component in a given tumour, though one can usually find all three elements on careful search. Rarely, Wilms' tumour can be biphasic or even monophasic. The prognosis of a given tumour appears to correlate with the histological elements. Stromal-predominant Wilms' tumour is known to show poor response to radio-/chemotherapy, whereas blastemal component shows good response. The existence of *nephrogenic rests* in normal kidneys has long been interpreted as residual embryonal elements that have failed to differentiate normally. There is a significant association between the presence of nephrogenic rests in the kidneys and Wilms' tumour (28–40%). This again suggests that Wilms' tumour may be a case of renal maldevelopment [7, 9].

A constellation of other findings may be found in children with Wilms' tumour which is further evidence of a genetic aetiology. For instance, children who develop **W**ilms' tumour in association with **a**niridia, **g**enitourinary anomalies and mental **r**etardation (termed WAGR syndrome) have a deletion at the WT1 gene locus (Fig. 49.3) [5, 6]. Children with Denys-Drash syndrome (characterized by pseudohermaphroditism, progressive glomerulopathy and Wilms' tumour) also have a point mutation of the WT1 gene [5, 6]. Children with Beckwith-Wiedemann syndrome (exomphalos, macroglossia, hyperinsulinemic hypoglycaemia) have DNA loss at the WT2 gene locus and have such a high risk of developing Wilms' tumour or hepatoblastoma that a screening programme in early childhood has been instituted to diagnose these tumours at an early stage (Fig. 49.4) [6].

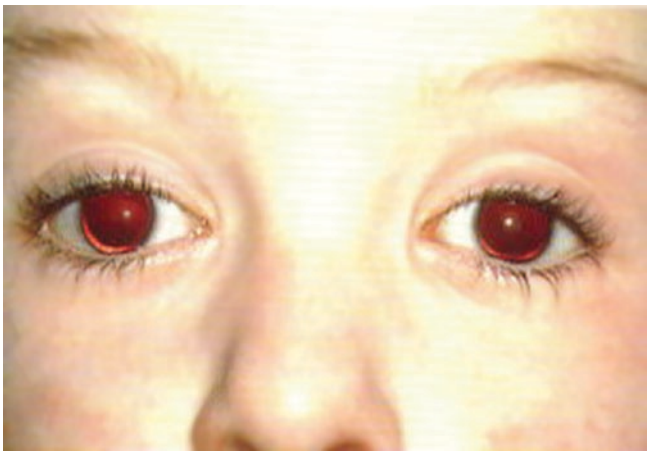

Fig. 49.3 Clinical picture of aniridia

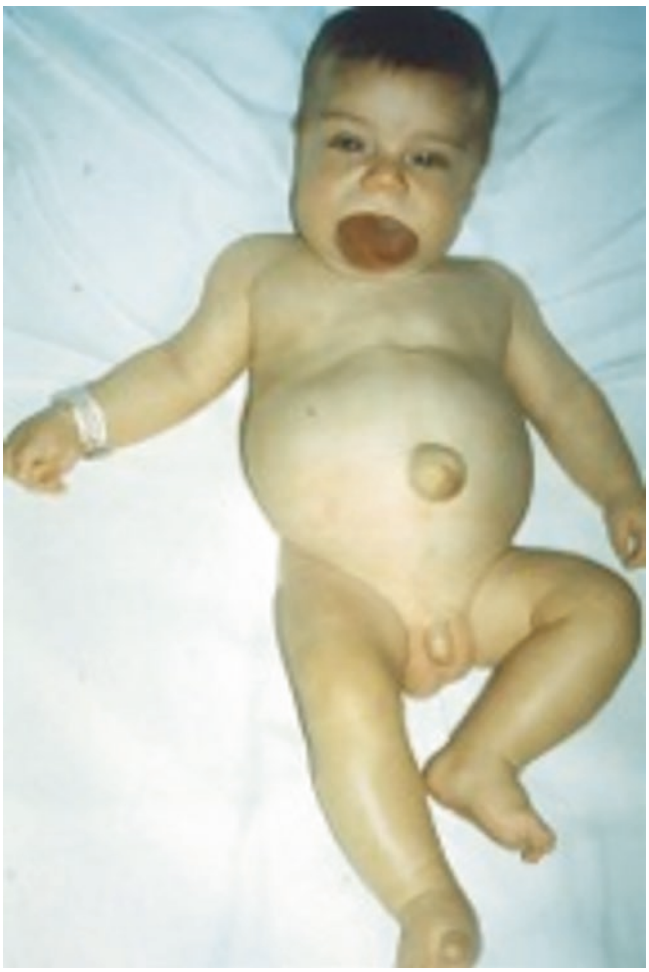

Fig. 49.4 Clinical picture of Beckwith-Wiedemann syndrome

Germ Cell Tumours

Germ cell tumours (GCTs) occur in both gonadal and extragonadal sites, with extragonadal (and testicular tumours) predominating in children younger than 3 years and gonadal (testicular and ovarian) tumours predominating during and after puberty [5]. It is thought that extragonadal GCTs arise when there is aberrant migration or deposition of germ cells along the path of migration or in abnormal locations. Therefore, GCTs are found in the sacrococcygeal area, mediastinum, retroperitoneum, pineal area of the brain as well as the ovary and testis. Malignant transformation may then occur at any of

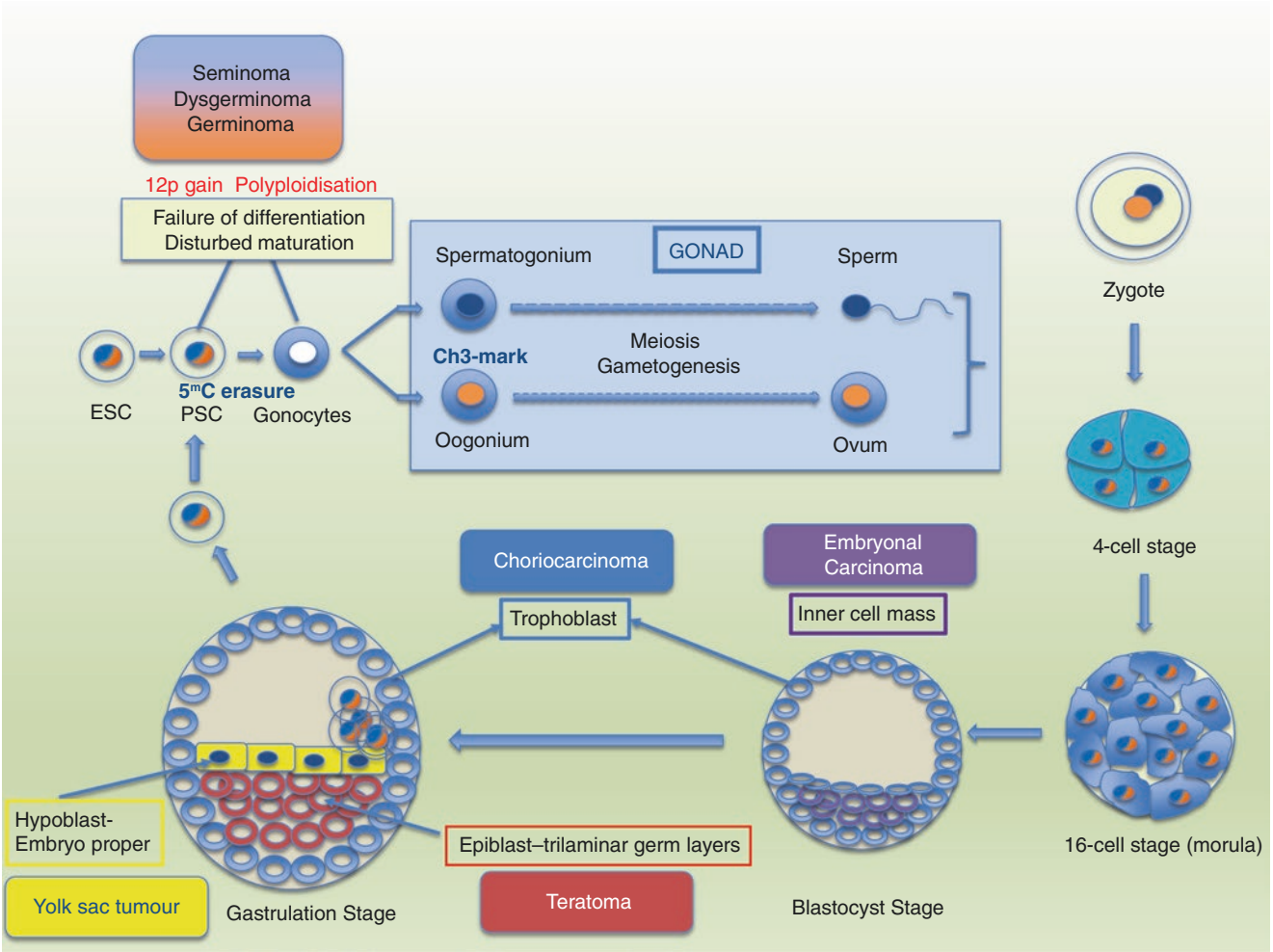

Fig. 49.5 Schematic diagram depicting development of germ cell tumours

these sites with the broad spectrum of types reflecting the totipotent/pluripotent nature of germ cells [7].

Embryological basis of germ cell tumours: Primordial germ cells (PGCs) originate from embryonal stem cells near the allantois of the embryonic yolk sac endoderm. To remove the parental genetic imprints, they undergo epigenetic erasure of $5^{m}C$ methylation. By the fifth gestational week, these PGCs with methylation erasure migrate through the mesentery to the gonadal ridge. Here the PGCs undergo genetic reprogramming by undergoing de novo methylation to acquire 'methylation mark' and become committed to gender-specific gametogenesis and eventually differentiate to form the gonads [5, 7] (Fig. 49.5).

Genetic factors (polyploidisation or 12p gain) transform these germ cells to form *intra-tubular germ cell neoplasia (ITGCN)*. If the transformed totipotent PGCs (with $5^{m}C$ methylation erasure) undergo clonal proliferation without further differentiation, it gives rise to seminoma (in males) and dysgerminoma (in females). If the transformed pluripotent PGCs (with de novo methylation) show differentiation towards inner cell mass, it gives rise to embryonal tumour. If the germ cells show intra-embryonic differentiation, it gives rise to teratoma (epiblastic differentiation/future embryo proper) or to endodermal sinus tumour (hypoblastic differentiation/future yolk sac). Extraembryonic, trophoblastic differentiation gives rise to choriocarcinoma [7, 10] (Fig. 49.5).

A teratoma is composed of representative tissue from each of the three germ layers of the embryonic disc (ectoderm, endoderm and mesoderm) [5] (Fig. 49.6). Mesodermal components such as fat, cartilage, bone and muscle are particularly common although endodermal tissues also commonly result in cystic structures lined by squamous, cuboidal or flattened epithelium [5, 7]. Most paediatric teratomas are mature (benign) with little tendency to undergo malignant degeneration [6]. Foci of malignant yolk sac tumour may be indicated by elevated serum alpha-fetoprotein (AFP) levels which may be used as a tumour marker [7]. The sacrococcygeal region is the

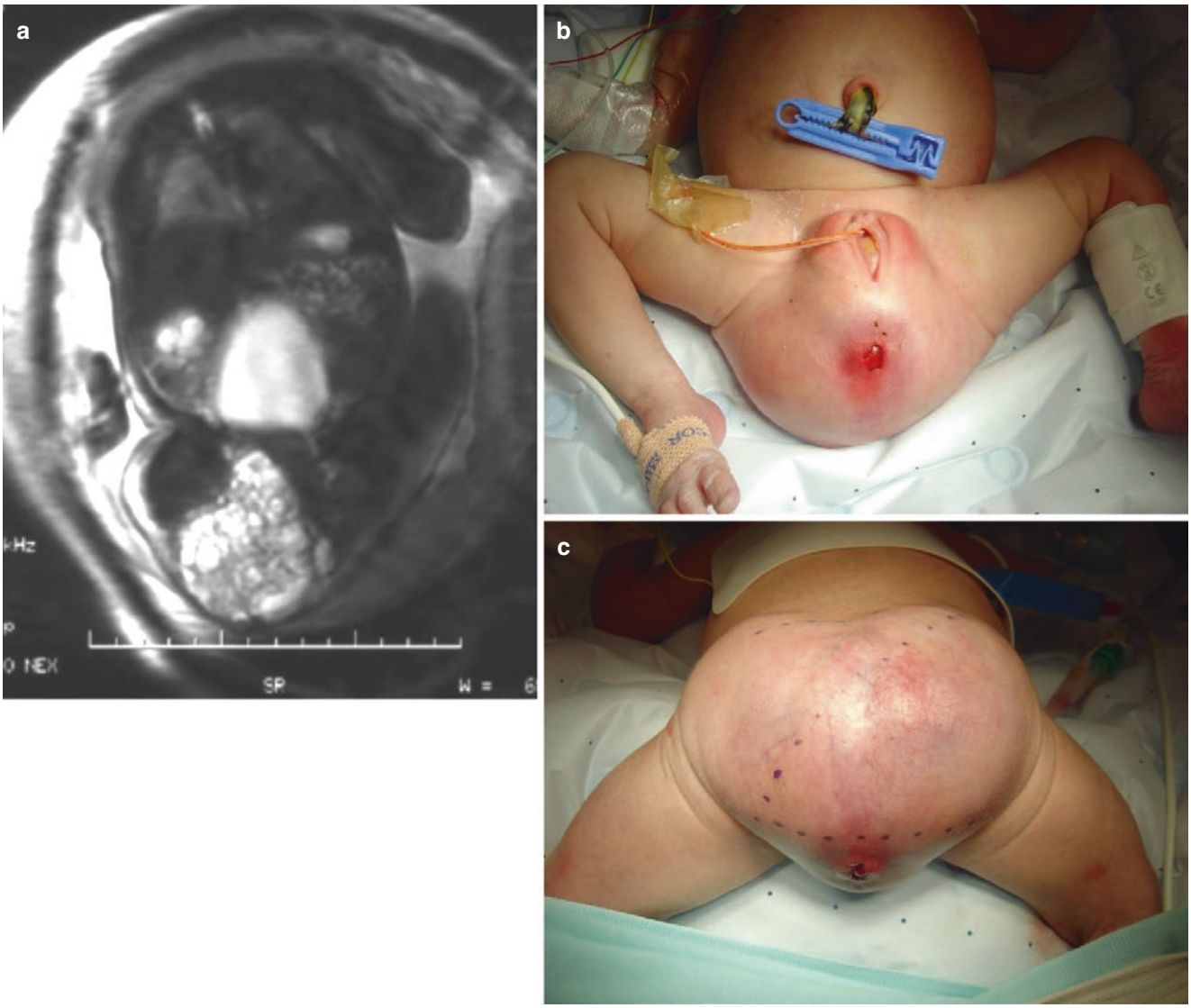

Fig. 49.6 (**a**) shows a large pelvic mass in the prenatal MRI at 32 gestational weeks. (**b**, **c**) show a sacrococcygeal teratoma

most common extragonadal location of teratomas and is usually diagnosed in the first year of life (Fig. 49.7) [6]. Over half of paediatric ovarian tumours are teratomas which often present with pain when large tumours cause torsion of the ovary. Teratomas are also the most common testicular neoplasm in childhood [6].

Germinomas (often referred to as *seminoma* when found in the testis or *dysgerminoma* when in the ovary) may secrete β-hCG (human chorionic gonadotropin) which may also be used as a serum marker of malignancy [7]. Germinoma is the predominant malignancy found in dysgenetic gonads and undescended testes. Boys with undescended testes are likely to have a threefold increased risk of later development of testicular cancer compared to other boys [11]. Gonadoblastoma is a rare tumour which is usually found in dysgenetic gonads of phenotypic females who have a fragment of the Y chromosome, and because of this risk, prophylactic gonadectomy may be recommended for these girls [5, 7].

Neuroblastoma

Neuroblastoma is the most common malignancy diagnosed in infancy and is remarkable for its broad spectrum of clinical behaviour [5, 6]. The clinical course can be variable, as spontaneous regression or maturation may occur in young infants, whilst other children develop progressive neuroblastoma with a dismal prognosis [6]. Neuroblastoma cells are found in the adrenal gland in about 1 in every 40 neonates who die of other causes; yet clinical neuroblastoma presents in only approximately 1 in 10,000 children [6].

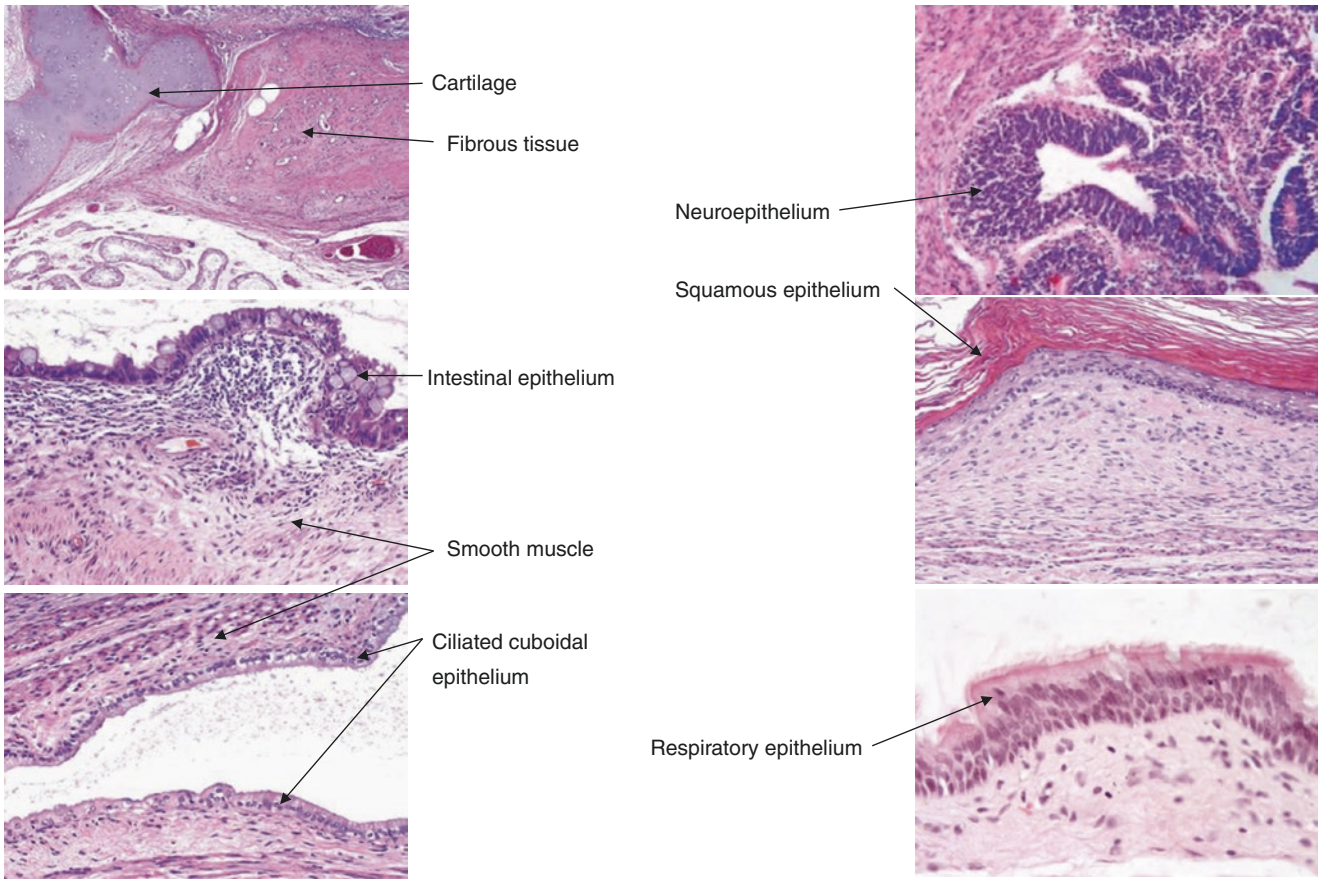

Fig. 49.7 Histology of teratoma shows divergent differentiation towards all three germ layers

In an attempt to identify early cases of neuroblastoma amenable to cure, mass screening has been attempted in several countries by evaluating urinary catecholamine metabolites in infants. Unfortunately, tumours detected by screening apparently seem to be the group which would have regressed spontaneously, whilst there is no reduction in the incidence of neuroblastoma-related deaths [6]. Clinical presentation is variable, depending on the site of primary and metastatic tumours causing a mass effect with over half having an abdominal mass. Catecholamines released by these neuroblastoma cells may be detected when excreted in urine and help with diagnosis [5, 6].

Embryological basis of neuroblastoma: Primitive neuroblasts can be identified in the foetal adrenal gland from 10 weeks of gestation. Antenatal ultrasound of the adrenal glands has identified neuroblastoma in numerous reports. Neuroblast cells are derived from primordial neural crest cells which migrate from the mantle layer of the embryonic spinal cord [4]. They populate tissues such as the adrenal medulla and sympathetic ganglion chain, and hence, neuroblastoma may occur at any site from the neck to the pelvis. The fate of these neuroblasts may be to regress spontaneously, mature by differentiation to benign ganglioneuroma or progress to malignant neuroblastoma [7]. The cell pathway is determined by complex molecular signalling mechanisms determined by genetic abnormalities (Fig. 49.8. Spectrum of neuroblastic tumours).

N-myc is a transcription factor related to a proto-oncogene found at the chromosome 2p24 locus, which has become one of the most important biological factors for the prognosis [5–7, 11]. When there are ≥10 copies of N-myc, as detected by fluorescent in situ hybridization (FISH), it is considered to represent genomic amplification which confers a poor prognosis. N-myc amplification promotes proliferation of neuroblasts and their transformation to neuroblastoma by preventing terminal differentiation and successful exit from the cell cycle [6]. Approximately 30% of all neuroblastoma cases are found to be N-myc-amplified [12].

Hepatoblastoma

Hepatoblastoma is the most common hepatic malignancy in children below 3 years of age. A number of genetic predispositions increase the risk of hepatoblastoma, and the most important ones include Beckwith-Wiedemann syndrome and familial adenomatous polyposis (FAP) [5–7].

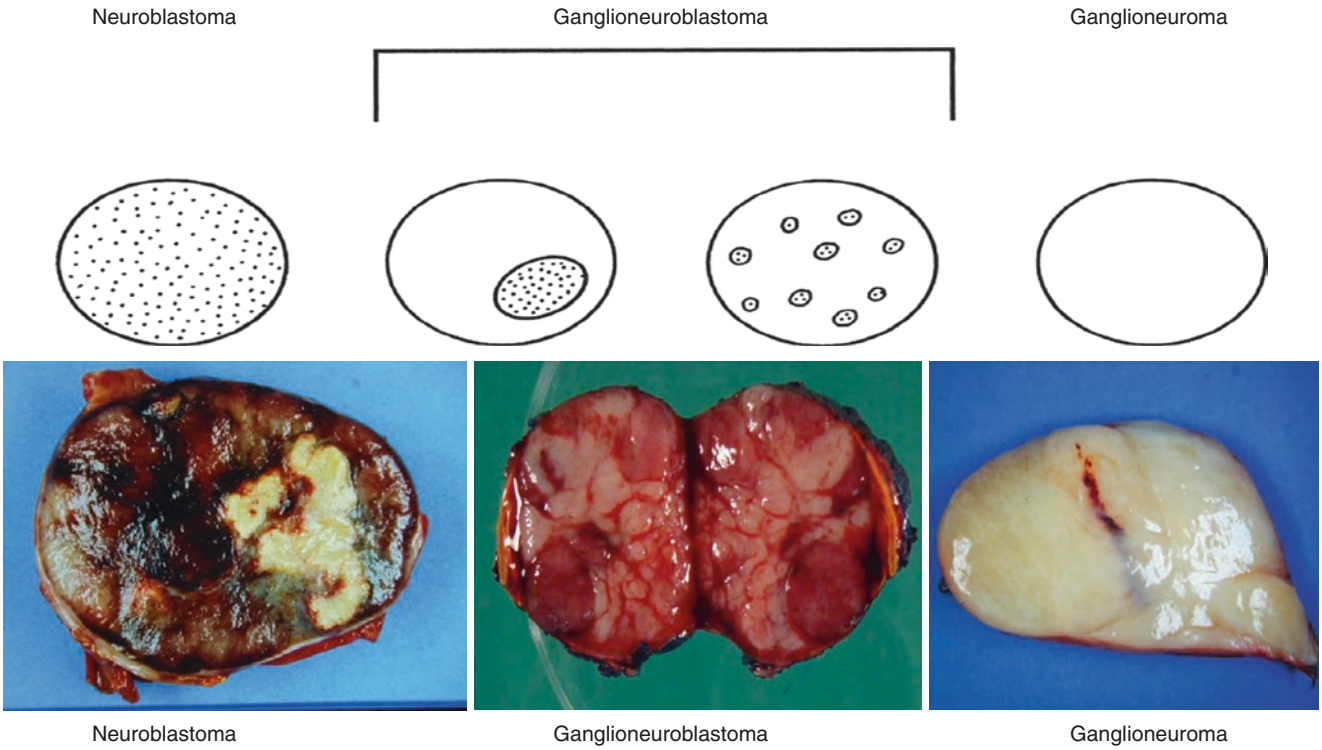

Fig. 49.8 Spectrum of neuroblastic tumours

Embryological basis of hepatoblastoma: Hepatoblastoma is an embryonal tumour that recapitulates various developmental stages of liver development. Many developmental pathways (Wnt, Notch, Shh, c-MET, etc.) play an important role in hepatic development. Wnt signalling is one such pathway that is crucial for embryonal liver development and is frequently dysregulated in hepatoblastoma [13, 14]. This is reflected by the fact that there is an increased incidence of hepatoblastoma in families with FAP, where there is a germline mutation of APC gene [14]. APC plays a central role in the proteasomal degradation of beta-catenin, a powerful nuclear transcription factor and effector of Wnt pathway, which promotes cell proliferation, migration, invasion and cell survival [14]. Thus, constitutional activation of Wnt pathway confers a proliferation advantage to tumour cells in hepatoblastoma (Fig. 49.9. Schematic diagram of development of hepatoblastoma).

Histologically, hepatoblastoma can show a diverse range of differentiation towards epithelial (foetal, embryonal, mixed foetal/embryonal, small cell undifferentiated), mesenchymal or mixed epithelial/mesenchymal components. The different subtypes possibly reflect proliferation (following developmental arrest) rather than differentiation into next stage [7]. Recent research suggests that activation of specific developmental pathways at different stages of liver development may correlate with the degree of differentiation in various histologic subtypes [13, 14]. Wnt activation is present in embryonal and in mixed epithelial/mesenchymal subtypes, whereas Notch pathway activation is seen in foetal subtype [13, 14]. Small cell undifferentiated subtype is genetically distinct from others and shows aberrations in human SMARCB1 gene, causing them to show characteristic loss of INI-1 staining on immunohistochemistry, a feature that it shares more closely with malignant renal rhabdoid tumour and the CNS atypical teratoid/rhabdoid tumours including very dismal prognosis. Apart from pure foetal subtype, where surgery alone may suffice and enjoys a very good prognosis, other histological subtypes do not have any significant bearing on the overall clinical outcome.

Rhabdomyosarcoma

Malignancies that arise from connective tissues (mesodermal origin) in the body are called sarcomas. They may arise from tissues such as muscle, adipose tissue, fascia, blood vessels, nerves, bones, synovium or blood vessels. Sarcomas that display differentiation towards the skeletal muscle are

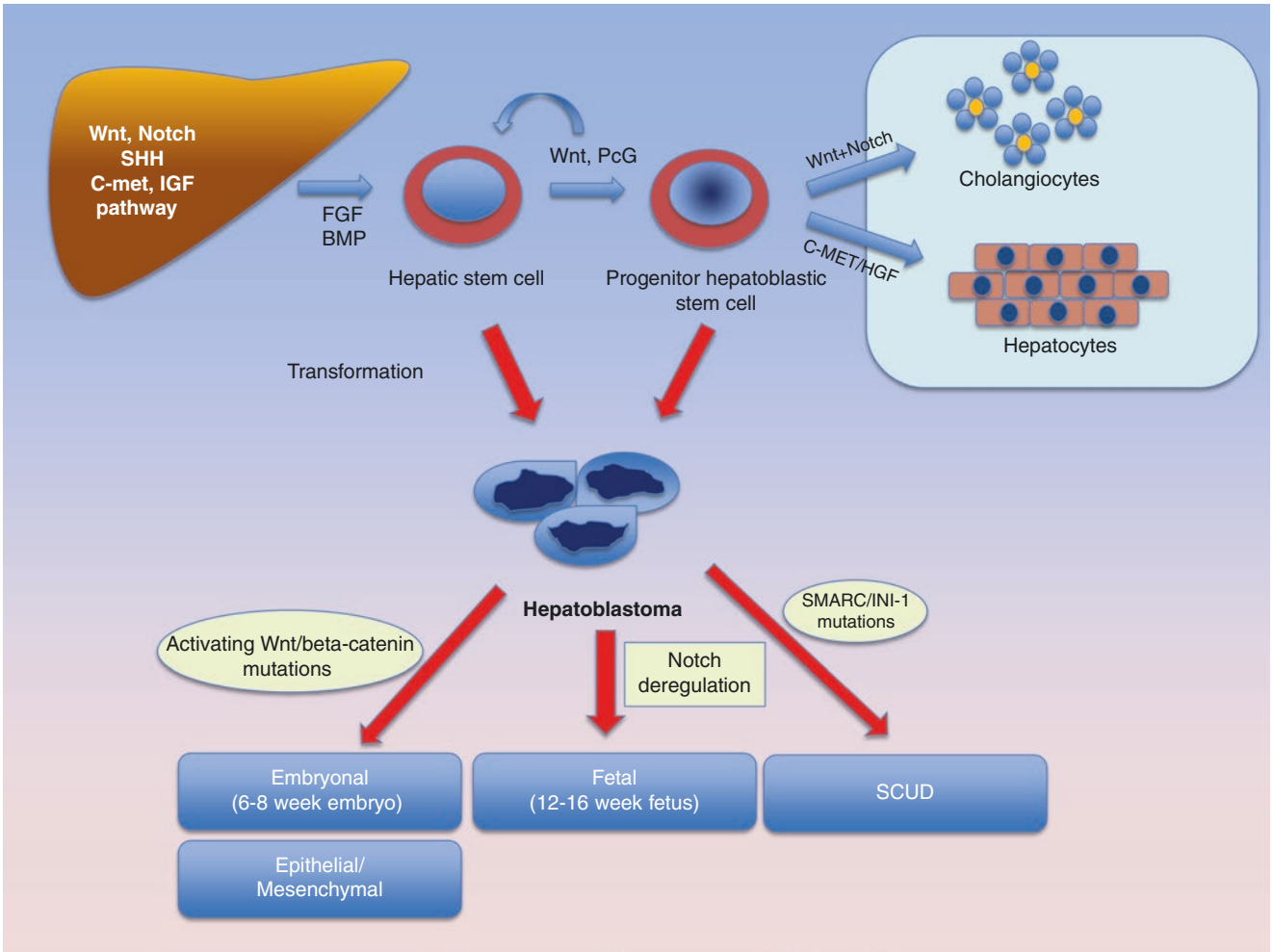

Fig. 49.9 Schematic diagram to show the development of the various subtypes of hepatoblastoma

collectively known as rhabdomyosarcoma (RMS). There principally are two types of RMS—embryonal and alveolar. These subtypes may essentially reflect the developmental stages of the skeletal muscle [7, 15].

Embryonal RMS usually affects children <5 years of age and tends to occur in the head and neck region and the genitourinary organs. Botryoid and spindle cell RMS are considered to be further subtypes of embryonal RMS that tend to have a better prognosis than the conventional embryonal RMS. Alveolar RMS is a more aggressive tumour with a poorer prognosis than embryonal RMS and occurs mostly in the trunk and extremities [6, 7, 15].

Embryological basis of RMS: The uncommitted mesodermal stem cells at around 6–8 gestational weeks under the influence of Pax3/Pax7 genes differentiate asymmetrically into cells, committed myogenic progenitor cells and primitive (multinucleated) myotubes [15]. The primitive myotubes, under the influence of MyoD and Myf5, differentiate further into rhabdomyoblasts. Myogenin and Myf6 promote differentiation of rhabdomyoblasts into maturing myotubes and finally into mature skeletal muscle fibres. The committed myogenic progenitor cells reside as 'satellite cells' in the mature skeletal muscle and serve as source of regeneration by forming more multinucleated myotubes [15] (Fig. 49.10).

Alveolar RMS is characterized by Pax3/Pax7-FOXO translocation that causes uncontrolled proliferation at the stages of uncommitted mesodermal stem cells or satellite cells within the maturing muscle tissue that may give rise to the alveolar RMS subtypes, respectively (similar to foetal muscle at 10 gestational weeks) [16]. Similarly, proliferation following arrest at the rhabdomyoblast and/or the maturing myotube stages may give rise to embryonal RMS (similar to foetal muscle at 6–7 gestational weeks) [15] (Fig. 49.10).

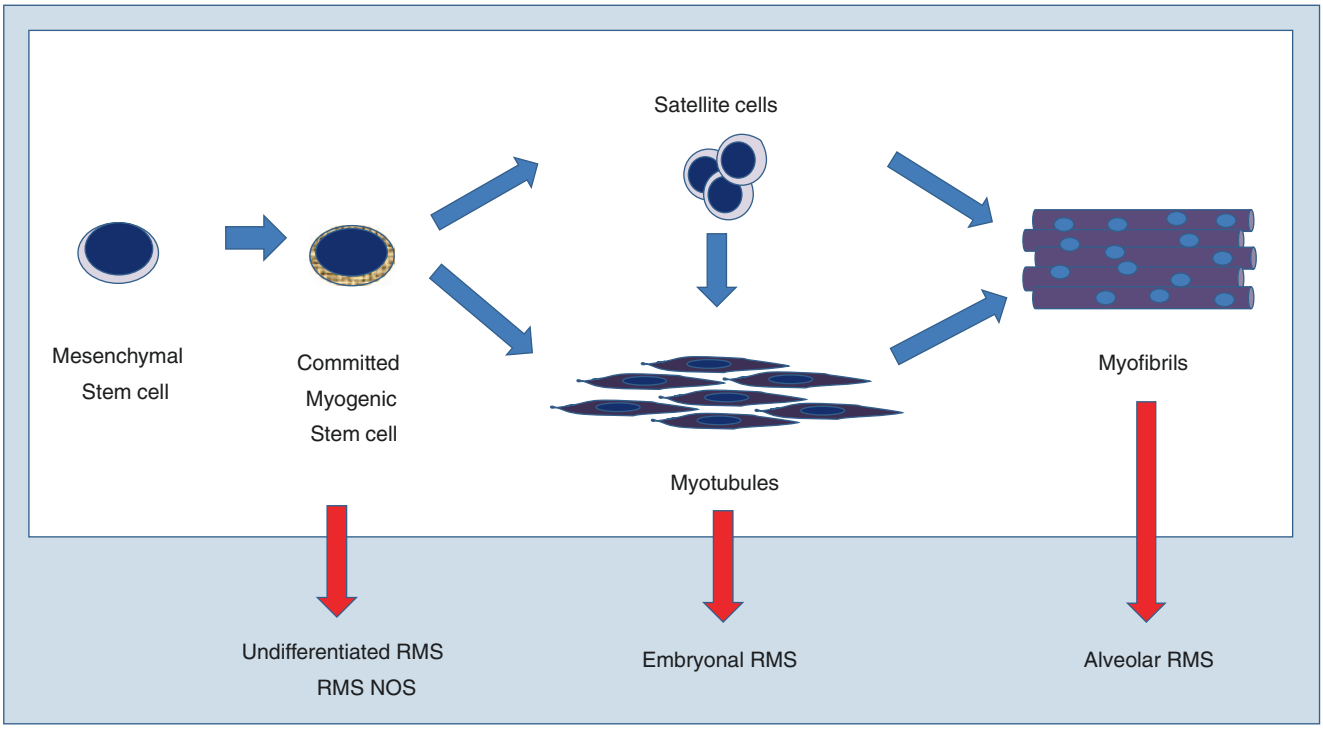

Fig. 49.10 Schematic diagram of development of rhabdomyosarcoma

The underlying genetics of embryonal RMS is not consistent though many show 11p15.5 aberrations; many show possible deregulation of multiple pathways involving IGF2, p53, Rb1, Wnt, Shh and Notch pathways [15–17].

Conclusion

The embryologic basis of tumours is demonstrated through the prism of these paediatric examples although this role can be extrapolated to the breadth of adult oncological practice. Although tumours presenting congenitally are rare, our inherited genotype has a major impact on our predisposition to the development of tumours and other conditions in later life.

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