Novel mutations target distinct subgroups of medulloblastoma

Medulloblastoma is a malignant childhood brain tumour comprising four discrete subgroups. Here, to identify mutations that drive medulloblastoma, we sequenced the entire genomes of 37 tumours and matched normal blood. One–hundred and thirty–six genes harbouring somatic mutations in this discovery set were sequenced in an additional 56 medulloblastomas. Recurrent mutations were detected in 41 genes not yet implicated in medulloblastoma; several target distinct components of the epigenetic machinery in different disease subgroups, such as regulators of H3K27 and H3K4 trimethylation in subgroups 3 and 4 (for example, KDM6A and ZMYM3), and CTNNB1–associated chromatin re–modellers in WNT–subgroup tumours (for example, SMARCA4 and CREBBP). Modelling of mutations in mouse lower rhombic lip progenitors that generate WNT–subgroup tumours identified genes that maintain this cell lineage (DDX3X), as well as mutated genes that initiate (CDH1) or cooperate (PIK3CA) in tumorigenesis. These data provide important new insights into the pathogenesis of medulloblastoma subgroups and highlight targets for therapeutic development.

The genomic landscape of medulloblastoma

To identify genetic alterations that drive medulloblastoma, we performed whole-genome sequencing (WGS) of DNA from 37 tumours and matched normal blood (discovery cohort). Tumours were subgrouped by gene expression (WNT subgroup, n = 5; SHH subgroup, n = 5; subgroup 3, n = 6; subgroup 4, n = 19; ‘unclassified’ (profiles not available), n = 2; Fig. 1, Supplementary Figs 1–3 and Supplementary Table 1). Validation of all putative somatic alterations including single nucleotide variations (SNVs), insertion/deletions (indels) and structural variations (SVs) identified by CREST\(^*\), was conducted for 12 tumours using custom capture arrays and Illumina-based DNA sequencing (Supplementary Table 2). Putative coding alterations and SVs were validated in the remaining 25 discovery cohort cases by polymerase chain reaction (PCR) and Sanger-based sequencing. Mutation frequency was determined in a separate ‘validation cohort’ of 56 medulloblastomas (WNT subgroup, n = 6; SHH subgroup, n = 8; subgroup 3, n = 11; subgroup 4, n = 19; unclassified, n = 12; Fig 1 and Supplementary Table 1).

WGS of the discovery cohort detected 22,887 validated or high-quality somatic sequence mutations (SNVs and indels), 536 validated or curated SVs, and 5,802 copy number variations (CNVs); 92%
concordant with 6.0 SNP mapping arrays; Supplementary Tables 3–6 and Supplementary Figs 4–7). In all but five tumours with the highest mutation rates, >50% of SNVs were C→T/G→A transitions (Supplementary Fig. 8). The mean missense:silent mutation ratio was 3.6±1 and 40% of all missense mutations were predicted to be deleterious, suggesting a selective pressure for SNVs that affect protein coding (Supplementary Table 5). Global patterns of total SNVs and amplifications varied significantly among medulloblastoma subgroups, even when corrected for age and sex, supporting the notion that these tumours are distinct pathological entities (Fig. 1 and Supplementary Fig. 6). Custom-capture-based analysis of the allele frequency of all somatic mutations in 12 medulloblastomas allowed us to predict the ancestry of certain genetic alterations, suggesting that aneuploidy precedes widespread sequence mutation in medulloblastomas with highly mutated genomes (Supplementary Figs 9–11).

Novel CNVs and SVs are rare in medulloblastoma

The repertoire of focally amplified or deleted genes seems to be very limited in medulloblastoma. We detected expected gains of MYCN, PTEN, and MYC in subgroup-4 tumours (nos 11–13) and one unclassified tumour deleted DDX31, AK8 and TSC1 at chromosome 9q34.14 in concert with OTX2 amplification, suggesting that these alterations are cooperative (P < 0.0005, Fisher’s exact test). The breakpoint in this deletion occurs in DDX31, and two samples contained a missense mutation (subgroup 4, no. 15) and complex rearrangement (unidentified case SJMB026) in this gene, suggesting that DDX31 is the target of these alterations (Supplementary Fig. 14).

Over 50% of SVs detected by WGS broke the coding region of at least one gene, but less than 2% (n = 6/314, excluding two tumours with excessive SVs) encode potential in-frame fusion proteins (Supplementary Fig. 15); none affect the same gene or signal pathway. Therefore, fusion proteins are likely to be an uncommon transforming mechanism in medulloblastoma.

Although germline mutations in TP53, PTCH1, APC and CREBBP predispose to medulloblastoma11–14, only 23 mutations previously associated with cancer were detected in discovery cohort germ lines. Only one of these—in a known case of Turcot’s syndrome—was accompanied by a somatic mutation (germline APC Y935*/somatic deletion; WNT subgroup no. 11; Supplementary Table 8). Thus, inherited forms of medulloblastoma seem to be rare in our cohort.

Novel mutations in medulloblastoma subgroups

Because SVs and CNVs are unlikely to drive most medulloblastomas, we investigated whether recurrent (more than two samples) somatic SNVs and/or indels might target discrete genes and pathways. This analysis identified 49 genes, across all 93 tumours, which were targeted by non-silent, recurrent, somatic mutations; 84% (n = 41/49) have not yet been implicated in medulloblastoma (Supplementary Tables 9 and 10). Several of these congregated in disease subgroups and converged on specific cell pathways (Fig. 1, Supplementary Fig. 8 and Supplementary Table 11).

Histone methylation is deregulated in subgroups 3 and 4

The H3K27 trimethyl mark (H3K27me3) represses lineage-specific genes in stem cells15 (Supplementary Fig. 8). H3K27me3 is written by the histone modifier TRRAP and its parologue KDM6A and its paralogue KDM6B and KDM5A (chromosome Xp11.2) or KDM6A and its paralogue UTY (chromosome Yq11), occurs in adult female and male cancers, respectively24.

Hypergeometric distribution analyses revealed selective mutation of histone modifiers in subgroup-3 and -4 medulloblastomas (Supplementary Table 11). Six subgroup-4, one subgroup-3, and
one unclassified medulloblastoma contained novel inactivating mutations in KDM6A (Figs 1 and 2 and Supplementary Figs 8 and 16). The single female with a KDM6A splice-site mutation showed a deletion of the second allele that escapes X inactivation\(^\text{26}\) (subgroup 4, no. 15), and 57% \((n = 4/7)\) of KDM6A-mutant male medulloblastomas deleted chromosome Y, compared with only 6% \((n = 3/51)\) of male, KDM6A wild-type tumours \((P < 0.005, \text{ Fisher’s exact test; Fig. 1})\).

Thus, a two-hit model of KDM6A-UTY tumour suppression seems to operate in subgroup-4 medulloblastomas. Notably, mutations in six other KDM family members \((\text{KDM1A, KDM3A, KDM4C, KDM5A, KDM5B and KDM7A})\) were detected exclusively in subgroup-3 and -4 tumours, implicating broad disruption of lysine demethylation in these medulloblastomas (Fig. 1, Supplementary Table 11 and Supplementary Fig. 16).

Subgroup-3 and -4 medulloblastomas also gained and overexpressed EZH2 \((\text{chromosome 7q35-34})\), which writes H3K27me3, and contained novel inactivating mutations in effectors and regulators of \(\text{H3K27me3}\) (EZH2 upregulation) or preserving \(\text{H3K4me3}\) (Supplementary Fig. 16). The carboxy terminus of CTNNB1 then recruits a series of protein complexes that remodel chromatin and promote transcription at WNT-responsive genes (Supplementary Fig. 8). These include: histone acetyltransferases (for example, CREBBP and TRRAP–TIP60 complexes)\(^\text{28,29}\); ATPases of the SWI/SNF family (for example, SMARCA4)\(^\text{30}\); and the mediator complex that coordinates RNA polymerase II placement (for example, MED13)\(^\text{31}\). As expected, relatively low levels of H3K27me3 were detected in LRLPs and committied GPNPs, which generate WNT- and SHH-subgroup medulloblastomas, respectively\(^\text{3,5}\), potentially explaining why mutations that preserve this epigenetic mark are absent from these tumours. We recently showed that subgroup-3 medulloblastomas arise from a rare fraction of cerebellar progenitors\(^\text{6}\). We are currently investigating whether these progenitors are found among the H3K27me3-positive cells seen in the external germinal layer (Fig. 2b).

**Novel mutations in WNT-subgroup medulloblastomas**

WNT-subgroup medulloblastomas contained mutations in epigenetic regulators that are different to those seen in subgroup-3 and -4 disease. CTNNB1, the principal effector of the WNT pathway, forms a transcriptional factor with the T-cell factor/lymphoid enhancer factor factor \((\text{TCF/LEF})\)\(^\text{28}\). The carboxy terminus of CTNNB1 then recruits a series of protein complexes that remodel chromatin and promote transcription at WNT-responsive genes (Supplementary Fig. 8). These include: histone acetyltransferases (for example, CREBBP and TRRAP–TIP60 complexes)\(^\text{28,29}\); ATPases of the SWI/SNF family (for example, SMARCA4)\(^\text{30}\); and the mediator complex that coordinates RNA polymerase II placement (for example, MED13)\(^\text{31}\). As expected, >70% \((n = 8/11)\) of WNT-subgroup medulloblastomas contained mutations that stabilize CTNNB1 (Fig. 1 and Supplementary Fig. 8; \(P < 0.0001, \text{ Fisher’s exact test})\)\(^\text{28,31}\). A single subgroup-3 case \((\text{no. 5})\) gains KDM7A and KDM6A-CHD7 and ZMYM3 mutations were confined to subgroups 3 and 4, and clustered in samples with sub-median EZH2 expression levels (Fig. 2a; \(P < 0.05, \text{ Fisher’s exact test})\). These data suggest that subgroup-3 and -4 medulloblastomas retain a stem-like epigenetic state by aberrantly writing \((\text{EZH2 upregulation})\) or preserving \((\text{KDM6A-UTY inactivation})\) H3K27me3, or disrupting H3K4me3 associated transcription \((\text{CHD7 and ZMYM3 inactivation})\).

Finally, we looked to see if the differential expression of H3K27me3 among medulloblastoma subgroups reflects ancestral chromatin marking in the progenitors that generate these tumours (Fig. 2b). Relatively low levels of H3K27me3 were detected in LRLPs and committed GPNPs, which generate WNT- and SHH-subgroup medulloblastomas, respectively\(^\text{3,5}\), potentially explaining why mutations that preserve this epigenetic mark are absent from these tumours. We recently showed that subgroup-3 medulloblastomas arise from a rare fraction of cerebellar progenitors\(^\text{6}\). We are currently investigating whether these progenitors are found among the H3K27me3-positive cells seen in the external germinal layer (Fig. 2b).

![Figure 2](image-url)
also showed a mutation in CTNNB1, but this mutation has not been reported in cancer, did not upregulate nuclear CTNNB1 (Fig. 1) and is of unclear relevance. Remarkably, six WNT-subgroup medulloblastomas showed mutations in chromatin modifiers that are recruited to TCF/LEF WNT-responsive genes by CTNNB1 (Fig. 1 and Supplementary Fig. 8). Four WNT-subgroup tumours contained heterozygous missense mutations in the helicase domain of SMARCA4 (P < 0.002, Fisher’s exact test), two samples, including one with a SMARCA4 mutation (no. 5), contained nonsense mutations in CREBBP (WNT-subgroup enrichment, P < 0.02, Fisher’s exact test), and missense mutations in TRRAP and MED13 were detected in a single WNT-subgroup medulloblastoma each. Thus, in addition to stabilization of CTNNB1, the development of WNT-subgroup medulloblastoma may require disruption of chromatin remodelling at WNT-responsive genes.

A small number of WNT-subgroup medulloblastomas lack mutations in CTNNB1 or APC, suggesting that alternative mechanisms drive aberrant WNT signals in these tumours. Three WNT-subgroup medulloblastomas in our series contained wild-type CTNNB1 (nos 1, 10 and 11; Fig. 1). Sample no. 11 inactivated APC as the sole case of Turcot’s syndrome in our study, but this tumour and sample no. 10 also contained novel missense mutations in CDH1 (R63G, V329F; WNT-subgroup enrichment, P < 0.05, Fisher’s exact test; Fig. 1). CDH1 sequesters CTNNB1 at the cell membrane34, and mutations that disrupt this interaction promote WNT signalling in adult cancers35,36. The functional consequences of CDH1(R63G) and CDH1(V329F) remain to be determined, but their restriction to WNT-subgroup tumours, mutual exclusivity with CTNNB1 mutations, and adjacency to residues mutated in breast cancer (http://www.sanger.ac.uk/genetics/CGP/cosmic/), suggest they might promote aberrant WNT signals in medulloblastoma.

We showed previously in mice that mutant Ctnnb1 initiates WNT-subgroup medulloblastoma by arresting the migration of LRLPs from the embryonic dorsal brainstem to the pontine grey nucleus (PGN)3. Therefore, to test whether disruption of CDH1 might substitute for mutant CTNNB1 in medulloblastoma, we used short hairpin (sh)RNAs to knockdown Ctnnb1 in embryonic day (E)14.5 mouse LRLPs (Fig. 3a–c). Deletion of Cdh1 expression upregulated Tcf/Lef-mediated gene transcription in LRLPs and more than doubled their self-renewal capacity (Fig. 3b). Furthermore, in utero electroporation of LRLPs with Cdh1 shRNAs impeded their migration from the dorsal brainstem to the PGN with an efficiency similar to that of mutant Ctnnb1 (Fig. 3d, e; see Supplementary Methods). These data support the hypothesis that CDH1 suppresses the formation of WNT-subgroup medulloblastoma by regulating WNT-signals in LRLPs.

WNT-subgroup medulloblastomas were also enriched for novel, recurrent somatic missense mutations in the DEAD-box RNA helicase DDX3X at chromosome Xp11.3 (P < 0.0001, Fisher’s exact test; Fig. 1). DDX3X regulates several critical cell processes including chromosome segregation37, cell cycle progression38, gene transcription and translation39. Previously reported cancer-associated mutations in DDX3X disrupt the ATPase activity of the protein, but seven of eight mutations identified in our series clustered in the DEAD-box domain (Supplementary Information and Supplementary Fig. 8). Structural modelling predicts that these mutations interfere with nucleic acid binding, possibly altering specificity and/or affinity for RNA substrates, rather than inactivating DDX3X (Supplementary Figs 17–22). Indeed, the wild-type allele of DDX3X that escapes X inactivation35 was retained by two of three DDX3X-mutant female medulloblastomas, and knockdown of Ddx3x halved the self-renewal rate of mouse LRLPs, suggesting that this protein is important for the proliferation and/or maintenance of the LRP lineage (Fig. 3b).

To understand better the role of DDX3X in WNT-subgroup medulloblastoma, we used our in utero migration assay to assess the impact of Ddx3x shRNAs, mutant Ddx3xT275M (identified in WNT-subgroup sample no. 9), or mutant Ddx3xG325E (WNT sample no. 8) on LRLPs. Remarkably, although Ddx3x shRNAs were expressed abundantly in E14.5 brainstem cells within 48 h of electroporation, ≤ 0.5% of Ddx3x-shRNA-positive cells were present by postnatal day (P)1, confirming the critical importance of this gene to maintain the LRP lineage (Fig. 3d, e). In contrast, mice electroporated with either mutant Ddx3xT275M or Ddx3xG325E consistently contained...
~50% more labelled cells at P1 than did controls, although these cells migrated normally (Fig. 3d, e and data not shown). Thus, mutations in DDX3X may contribute to WNT-subgroup medulloblastoma by increasing LRLP proliferation rather than perturbing the migration of their daughter cells. Notably, comparable knockdown in utero of Mill2, Gabrg1 and Kdm6a that were selectively mutated in non-WNT medulloblastomas had no apparent impact on LRLPs; supporting the value of our assay for assessing WNT-subgroup specific mutations and underscoring the importance of cell context for functional studies of genes mutated in cancer subgroups.

**PIK3CA mutations promote WNT-subgroup medulloblastoma**

Cancer-associated, activating mutations in PIK3CA were detected in a single case each of WNT-subgroup (PIK3CA(Q546K)), SHH-subgroup (PIK3CA(H1047R)) and subgroup-4 (PIK3CA(N345K)) medulloblastoma (Fig. 1 and Supplementary Fig. 23). Although PIK3CA mutations are common in adult cancers and reported in medulloblastoma, their role in tumorigenesis remains controversial. In particular it is not known if these mutations initiate or progress cancer. To test this, we generated mice that express a conditional allele of the Pik3caE545K mutation. Mice harbouring Pik3caE545K or Pik3caE545K and Tp53R172H, were bred with Blbp-Cre, which drives efficient recombination in LRLPs.43,64 Blbp-Cre/Pik3caE545K mice, with or without Tp53R172H, survived tumour free for a median of 212 days with no evidence of aberrant LRLP migration (Fig. 4a and data not shown). In stark contrast, 100% (n = 11/11) of Blbp-Cre;Ctnnb11lox/Ex3,Tp53R172H,Pik3caE545K mice developed WNT-subgroup medulloblastomas by 3 months of age; only 4% (n = 2/54) of Blbp-Cre;Ctnnb11lox/Ex3,Tp53R172H,Pik3caE545K mice without Pik3caE545K, or with Tp53R172H, survived tumour free for a median of 121 days with no evidence of aberrant LRLP migration (Fig. 4a and data not shown). In stark contrast, 100% (n = 11/11) of Blbp-Cre;Ctnnb11lox/Ex3,Tp53R172H,Pik3caE545K mice developed WNT-subgroup medulloblastomas by 3 months of age; only 4% (n = 2/54) of Blbp-Cre;Ctnnb11lox/Ex3,Tp53R172H,Pik3caE545K mice without Pik3caE545K, or with Tp53R172H, survived tumour free for a median of 121 days with no evidence of aberrant LRLP migration (Fig. 4a and data not shown). In stark contrast, 100% (n = 11/11) of Blbp-Cre;Ctnnb11lox/Ex3,Tp53R172H,Pik3caE545K mice developed WNT-subgroup medulloblastomas by 3 months of age; only 4% (n = 2/54) of Blbp-Cre;Ctnnb11lox/Ex3,Tp53R172H,Pik3caE545K mice without Pik3caE545K, or with Tp53R172H, survived tumour free for a median of 121 days with no evidence of aberrant LRLP migration (Fig. 4a and data not shown).

**SHH-subgroup medulloblastomas**

Four of thirteen SHH-subgroup medulloblastomas contained expected biallelic inactivating alterations in SUFU or PTCH1. What drives aberrant SHH signals in the remaining cases remains unclear. These tumours contained mutations in MLL2, TP53 and PTEN that have been reported previously in medulloblastoma; but these mutations occur in other subgroups and are not known to activate SHH signals. Two SHH-subgroup tumours (nos 11 and 12) contained identical novel T48M mutations in the GABA_A (γ-aminobutyric acid, subtype A) receptor, γ1, which is predicted to be deleterious (Fig. 1 and Supplementary Table 9). Disruption of GABA_A receptors can enhance neural stem cell proliferation,41 suggesting that these mutations might deregulate the proliferation of GNPs that generate SHH-subgroup medulloblastomas.

**Discussion**

We have identified several, new, recurrent, somatic mutations in specific subgroups of medulloblastoma. Alterations affecting EZH2, KDM6A, CHD7 and ZMYM3 seem to disrupt chromatin marking of genes in subgroup-3 and -4 tumours. Further epigenetic studies will be required to uncover the identity of these genes, but evidence suggests these may include OTX2, MYC and MYCN.42-44 As amplification of these genes was detected almost exclusively in subgroup-3 and -4 tumours that lacked mutations in KDM6A, CHD7 or ZMYM3, it is tempting to speculate that these genetic alterations target common transforming pathways. A recent study detected recurrent mutations in three other chromatin remodelers in medulloblastoma; SMARCA4, MIL and ML3, but this study did not include details of tumour subgroup. Here, we show that mutations in SMARCA4, CREBBP, TRRAP and MED13 are enriched in WNT-subgroup medulloblastomas; thereby uncovering potential cooperative mutations in chromatin remodelers and their binding-partner oncogene, CTNNB1. Thus, disruptions in the epigenetic machinery of medulloblastoma are likely to be subgroup specific and may cooperate with other oncogenic mutations. The low incidence of MIL2 mutations detected in our study relative to previous work probably reflects differences in study populations (see Supplementary Results).

Although medulloblastoma is more prevalent in males, especially subgroup-3 and -4 disease, the reason for this sex bias is unknown. One potential explanation is the location of medulloblastoma oncogenes or tumour suppressor genes on chromosome X. Three of the most recurrently mutated genes detected in our study are located on chromosome X, of which two (ZMYM3 and KDM6A) were observed almost exclusively in males. Mutation in these genes might explain some of the male sex bias in medulloblastoma. The third mutated X chromosome gene, DDX3X, is more likely to be a WNT-subgroup medulloblastoma oncogene. Three of four female medulloblastomas carried heterozygous mutations in DDX3X that escape X inactivation, and our functional data indicate that mutations in this gene provide a proliferative advantage to LRLPs that generate these tumours.

Our findings also have important implications for drug development. Inhibitors of the epigenetic machinery, especially those that maintain H3K27me3—for example, EZH2 methylase—may be useful treatments for subgroup-3 and -4 disease. These tumours include the most aggressive forms of medulloblastoma, for which treatment options are limited. Mutations that activate PIK3CA and DDX3X in WNT-subgroup tumours might also be targeted with novel therapeutic strategies.45,46 Future clinical trials of drugs that target these mutant proteins must recruit the appropriate patient populations, as we demonstrate that mutations show subgroup specificity in medulloblastoma. Our accurate mouse models of WNT-subgroup, SHH-subgroup and subgroup-3 medulloblastomas should help with future studies of the biological and therapeutic importance of the novel genetic alterations described in this study.

**METHODS SUMMARY**

Human tumour and matched blood samples were obtained with informed consent through an institutional review board approved protocol at St Jude Children’s Research Hospital. WGS and analysis of WGS data were performed...
as previously described\(^\text{28}\). Details of sequence coverage, custom capture and other validation procedures are provided in Supplementary Information (Supplementary Tables 12–15). Immunohistochemistry and immunofluorescence of human and mouse tissues were performed using routine techniques and primary antibodies of the appropriate tissues as described (Supplementary Methods). Medulloblastoma mRNA and DNA profiles were generated using Affymetrix U133v2 and SNP 6.0 arrays, respectively (Supplementary Methods). Real-time PCR with reverse transcriptase (RT–PCR) analysis of genes targeted in mouse LI-PSL by lentiviral transduction and described previously\(^\text{24}\), LI-PSLs were isolated and transduced with indicated lentiviruses in stem cell cultures or targeted in uto in shRNAs or mutant DNA sequences by electroporation as described\(^\text{34}\) (Supplementary Information). Mice harbouring a Cre-inducible Ptk3\(^\text{CreE1}\) allele were generated using homologous recombination: a lox-

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**Central Brain Tumor Registry of the United States.** Report of: primary brain tumors of the United States, 1999–1999. https://www.cbtrus.org/REports/ 2002/2002report.pdf (CBTRUS, 2006).

---

**Taylor, M. D. et al.** Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 123, 465–472 (2012).

---

**Schietroma, J. et al.** Identification of granule neuron precursor identity is a critical determinant of progenitor cell competence to form SHH-induced medulloblastoma. Cancer Cell 14, 123–134 (2008).

---

**Yang, Z. J. et al.** Medulloblastoma can be initiated by deletion of Patched in lineage-restricted progenitors or stem cell systems. Cancer Cell 13, 135–145 (2008).

---

**Powell, G. et al.** Subtypes of medulloblastoma have distinct developmental origins. Nature 468, 1095–1099 (2010).

---

**Kawasumi, D. et al.** A mouse model of the most aggressive subgroup of human medulloblastoma. Nature Cell Biol. 12, 169–180 (2012).

---

**Mulhem, R. K. et al.** Neurogenic consequences of risk-adapted therapy for childhood medulloblastoma. J. Clin. Oncol. 23, 5511–5519 (2005).

---

**Wang, J. et al.** CREST maps somatic structural variation in cancer genomes with high-resolution DNA rearrangements. Nature 488, 855–849 (2011).

---

**Castellino, R. C. et al.** Hoxc8 haploinsufficiency promotes tumorigenicity in a mouse model of medulloblastoma. PLoS ONE 5, e10849 (2010).

---

**Hahn, H. et al.** Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 85, 841–851 (1996).

---

**Malik, D. et al.** Germline p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250, 1233–1236 (1990).

---

**Hamilton, S. R. et al.** The molecular basis of Turcotte’s syndrome. N. Engl. J. Med. 332, 839–847 (1990).

---

**Taylor, M. D. et al.** Medulloblastoma in a child with Rubenstein-Taybi syndrome: case report and review of the literature. Pediatr. Neurosurg. 35, 235–238 (2001).

---

**Mikkelsen, T. S. et al.** Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. Nature 448, 553–560 (2007).

---

**Cao, R. et al.** Loss of histone H3 lysine 9 methylation in a colorectal cancer cell line. Cancer Res. 62, 5652–5654 (2002).

---

**Agger, K. et al.** DUX4 enhancer in Zeste/Esc complexes has a histone H3 methyltransferase activity that marks chromosomal Polycistronic sites. Cell 111, 185–196 (2002).

---

**Argers, K. et al.** TXR and JMU3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 449, 731–734 (2007).

---

**Schmitz, M. P. et al.** Genomic distribution of CHD7 on chromatin tracks H3K4 methylation patterns. Genome Res. 19, 590–601 (2009).

---

**Savagner, M. & Sauvageau, G.** Polycistron protein groups: multi-faceted regulators of somatic cells and cancer cells. Cell 129, 293–313 (2007).

---

**Morrin, R. D. et al.** Somatic mutations altering EZH2 (Ty641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Cancer Cell 12, 299–313 (2007).

---

**Kleer, C. C. et al.** EZH2 is a marker of aggressive breast cancers and promotes neoplastic transformation of breast epithelial cells. Proc. Natl Acad. Sci. USA 100, 11606–11611 (2003).

---

**Varambally, S. et al.** The polycomb group protein EZH2 is involved in progression of prostate cancer by repression of p21 and p27, androgen receptor-dependent and independent. Nature 438, 432–435 (2005).

---

**Mukhopadhyay, D. et al.** EZH2 is a master regulator of gene expression in normal and cancer cells. Nature 446, 799–804 (2007).

---

**Agger, K. et al.** DUX4 Enhancer of Zeste/ESC complexes has a histone H3 methyltransferase activity that marks chromosomal Polycistronic sites. Cell 111, 185–196 (2002).

---

**Argers, K. et al.** TXR and JMU3 are histone H3K27 demethylases involved in HOX gene regulation and development. Nature 449, 731–734 (2007).

---

**Schmitz, M. P. et al.** Genomic distribution of CHD7 on chromatin tracks H3K4 methylation patterns. Genome Res. 19, 590–601 (2009).

---

**Savagner, M. & Sauvageau, G.** Polycistron protein groups: multi-faceted regulators of somatic cells and cancer cells. Cell 129, 293–313 (2007).

---

**Morrin, R. D. et al.** Somatic mutations altering EZH2 (Ty641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Cancer Cell 12, 299–313 (2007).

---

**Kleer, C. C. et al.** EZH2 is a marker of aggressive breast cancers and promotes neoplastic transformation of breast epithelial cells. Proc. Natl Acad. Sci. USA 100, 11606–11611 (2003).

---

**Varambally, S. et al.** The polycomb group protein EZH2 is involved in progression of prostate cancer by repression of p21 and p27, androgen receptor-dependent and independent. Nature 438, 432–435 (2005).

---

**Mukhopadhyay, D. et al.** EZH2 is a master regulator of gene expression in normal and cancer cells. Nature 446, 799–804 (2007).