p16

Stefano Serra, Runjan Chetty

Department of Anatomical Pathology, Laboratory Medicine Program, University Health Network and University of Toronto, Toronto, Ontario, Canada

Correspondence to

Professor Runjan Chetty, Department of Anatomical Pathology, Laboratory Medicine Program, University Health Network, Toronto General Hospital, Toronto, ON M5G 2C4, Canada; runjan.chetty@gmail.com

Accepted 16 June 2018
Published Online First
3 August 2018

ABSTRACT

The *p16* gene belongs to *INK4* family of genes and is made up of four members: *p16^{INK4A}*, *p15^{INK4B}*, *p18^{INK4C}* and *p19^{INK4D}*, all of which share biological properties, namely, inhibition of cell growth and tumour suppression. After *p53*, *p16* is the second most common tumour suppressor gene. It has been regarded as the familial melanoma gene. Immunohistochemistry for *p16* has a well-defined role in distinct pathological scenarios. It is used to distinguish desmoplastic melanoma from reactive fibrous proliferation, with former showing strong nuclear positivity. In other types of melanoma, *p16* protein expression is lost. Spitz nevi show retention of nuclear staining for *p16*. Benign mesothelial proliferations tend to retain nuclear *p16* immunoreactivity, while malignant mesotheliomas lose expression. However, *p16* fluorescent in-situ hybridisation analysis is recommended in the workup of malignant mesothelioma. Another common application of *p16* immunohistochemistry is as an indicator for human papillomavirus (HPV) infection and *p16* protein is overexpressed in HPV-associated tumours. In this context, *p16* immunopositivity should be strong, diffuse, nuclear or nuclear and cytoplasmic in location. Another use for *p16* is demonstration of *p16* immunopositivity in well-differentiated and dedifferentiated liposarcoma.

p16 is a tumour suppressor gene and goes by several names: MTS-1 (major tumour suppressor 1), *INK4a* (inhibitor of cyclin-dependent kinase 4a) or *p16^{INK4}* and *CDKN2A* (cyclin-dependent kinases inhibitor 2A). Its location at 9p21 is the site of loss of heterozygosity (LOH) in several malignancies and unsurprisingly, *p16* is thus implicated in several tumours¹.

The emphasis of this review will be centred on the lesions and tumours where *p16* and its protein expression are of diagnostic or potentially diagnostic value. Other tumours in which *p16* is abrogated will be covered briefly for completeness.

P16 GENE

The *INK4* family includes four members: *p16^{INK4A}*, *p15^{INK4B}*, *p18^{INK4C}* and *p19^{INK4D}*, which show analogous biological characteristics involved in inhibition of cell growth and in tumour suppression.^{1,2}

The tumour suppressor *p16* gene encodes proteins involved in the regulation of two fundamental cell cycle pathways, the *p53* and the *RB1* pathway.

The *INK4A* locus is localised in short arm of chromosome 9 at position band 21.3(9p21.3).³ Using alternative exons, the *p16* gene generates four transcriptional variants: *p16INK4A*, a cyclin-dependent

kinase inhibitor, *p14ARF* (alternative reading frame), which binds to MDM2,⁴ *p12* and *p16γ*.⁵

The structure of the *p16* gene includes exons *E1β*, *E1α*, *E2*, *E2γ* and *E3* (figure 1).

Exon *E1α* is located in the 5' region of the *p16* gene. *E2γ* is an alternatively spliced exon present between exon *E1α* and *E2*.⁶

The alternative splicing of *E1α* versus *E1β* to a shared additional exon present in the *INK4A* gene produces mRNAs that results in *E2* sequences with two diverse reading frames.

The transcript with *E1α* encodes *p16INK4a* while the transcript with *E1β* encodes *p14ARF* using an alternative AUG present in the *E1β* exon.

Thus, the splicing of exon *E1α* onto exons *E2* and *E3* generates the tumour suppressor *p16*.

The splicing of exon *E1β* onto exons *E2* and *E3* generates the tumour suppressor *p14ARF*. The sequence of exon *E1β* does not show any similarity with the one of exon *E1α*, which is transcribed using a different promoter. In addition, the reading frame of *p14ARF* is shifted by only a base pair; therefore, exons *E2* and *E3* have a new (ARF) with reference to that of *p16*. Consequently, *p14ARF* has a different structure compared with *p16* and shows diverse biological activities.⁵

The *p12* transcript has exon *E1α* in common with *p16*; using a different splice site present in intron 1, a continuous sequence of 274bp present in intron 1 is transcribed, before splicing onto *p16* exons *E2* and *E3*. The *p12* transcript seems to be present exclusively in human pancreas.⁵

p16γ transcript results from the splicing of the cryptic *E2γ*, present in *p16* intron 2, onto *p16* exons *E2* and *E3*. High levels of *p16γ* are detected in a primary T-ALL and a neuroblastoma cell line, while low levels are present in *p16*-expressing primary T-ALL and B-lineage-ALL samples.⁵

P16 PROTEIN STRUCTURE

The *p16* protein comprises four ankyrin repeats (AR) (figure 2). ARs are fairly preserved motifs of about 31–34 residues and consist of helix-turn-helix conformation. Adjacent ARs are connected together by loops of different length, which are orientated perpendicularly to the helical axes. The 4 AR motifs are arranged in a helix involved in the binding with the target proteins.

When *CDK6* binds to the cavity of *p16*, it exposes its catalytic cleft to *p16* inducing an electrostatic interaction between D84 of *p16* and R31 of *CDK6*, possibly causing a decrease in the kinase activity.

Cancer-related mutations at residue D84 of *p16* eliminated this inhibition causing unrepressed cell proliferation. *p16* protein could also prevent the action of *CDK4/6* by compromising the interaction with cyclin D. In *p16*, most of the residues, which

© Author(s) (or their employer(s)) 2018. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Serra S, Chetty R. *J Clin Pathol* 2018;71:853–858.

Figure 1 Schematic representation of the p16 gene (adapted from Li *et al*⁵).

interact with CDK4, are positioned in the second and third ARs and in the loop connecting these two ARs.⁵

P16 FUNCTION

p16 negatively regulates the pRb-E2F pathway during the cell cycle. In G0 and initial G1, pRb is hypophosphorylated and aggregates with E2F transcription factors, preventing E2F transcription factors to interact with the promoters of genes involved in proliferation (cyclin B1, dihydrofolate reductase, JUNB and others). When the cell proliferates, pRb undergoes increasing phosphorylation by CDK4 and CDK6 at the end of phase G1, driving the progression into S phase. The binding of p16 to CDK4 and CDK6 results in hypophosphorylation of pRb. In addition, p16 can disturb the complexes between CDK6/4 and non-p16 inhibitors, increasing the availability of non-p16 inhibitors, inhibition of CDK2 activity and increase of hypophosphorylated pRb. All these events result in arrest of cell proliferation at the G1/S limit. p16 interacts with TFIIF, an

essential transcription factor, preventing the phosphorylation of the C-terminal domain the subunit of RNA polymerase II in the C-terminus, resulting in cell cycle arrest.

p16 also interacts with JUNK1 and JUNK3 suppressing their kinase activity.

It has also been reported that p16 increases abnormally with ageing and that elevated levels of p16 induce senescence of progenitor cells and premalignant tumour cells.⁵

GENETIC ALTERATION

The frequency of *p16* inactivation in cancers range from 20% in breast cancer to 85% in pancreatic adenocarcinoma.⁵

The genetic alterations inducing inactivation of *p16* include point mutations, homozygous deletions, promoter hypermethylation and LOH.

The most frequent genetic alterations of *p16* are homozygous deletion and promoter hypermethylation.⁵

It seems that there is a predilection for a particular kind of *p16* alteration in certain tumour types: homozygous deletions of *p16* are more frequent in pancreatic adenocarcinoma (48%), while point mutations (30%) are recurrent in head and neck squamous cell carcinomas. In gastric adenocarcinoma, aberrant methylation represents the majority of *p16* alterations (34%), with p16 deletions or mutations being infrequent (0%–2%).⁵

The type of genetic alteration defines p16 functionality: homozygous deletions and promoter hypermethylation, typically, result in non-functional p16, while point mutations, particularly missense mutations, and in-frame small deletions, disrupt p16 structure and activity.⁵

In cancer, many of the missense mutations identified in p16 protein are mainly situated in the second, third and fourth ARs.⁵

These missense mutations are classified into four groups based on the mutagenic effect they have on the CDK4-inhibitory capacity, structural stability and protein integrity:

1. Missense mutations of residues implicated directly in the interaction with CDK4/6 are found on the concave aspect for CDK4 binding, resulting in preserved protein structure, similar conformational stability with prominent reduced CDK4-inhibitory activity.⁵
2. Missense mutations that affect residues indirectly implicated in CDK4 binding, affect the stability of p16/CDK4 inter-

Figure 2 Schematic representation of the p16 protein. Each ankyrin repeat consists of a helix-turn-helix (H-T-H) structure. The four H-T-H motifs are connected by three loops in beta and gamma turn structure (adapted from Byeon *et al*²⁴).

actions leading to a sensible reduction of CDK4-inhibitory function, analogous conformational stability with non-significant structural alterations.⁵

3. Missense mutations that involve residues implicated in forming the core structure of *p16* disrupt the entire structure of *p16* and consequently abolish its CDK4-inhibitory function.⁵
4. Missense mutations in the first, fourth ARs and the N and C-termini are implicated in binding to different factors such as TFIIF, JNKs and GRIM-19 or are involved in phosphorylation, without causing any obvious modifications in the inhibition of CDK4, stability or structure.⁵

REGULATION OF THE EXPRESSION

Regulation of *p16*

Transcription of *p16* shows several ways of control, many of which are regulated in several ways mostly via the regulatory elements located in the *p16* promoter.

Ets1 and Ets2 (E26 transformation-specific) transcription factors are targets of Ras-Raf-Mek pathway mediated through phosphorylation by MapKinases. A conserved Ets-binding site is located in the *p16* promoter containing a conserved Ets binding site. Ets1/Ets2 are positive regulators of *p16* expression.⁵

The latent membrane protein 1, encoded by the Epstein-Barr virus, suppresses the *p16* promoter by endorsing the exporting of Ets2 from the nucleus with inactivation of Ets2-mediated transactivation.⁵

An E-box (enhancer box) is a DNA response element that acts as a protein-binding site and regulates gene expression. Two E-box elements are present in the *p16* promoter.

E47 has a HLH domain which can regulate gene expression. On binding to the 2 E-boxes, E47 activates the expression of *p16* in ageing cells.⁵

On the other hand, the heterodimer E47-Id1 binds to E-box elements, leading to the inhibition of the *p16* promoter.⁵

Myc also binds to the E-boxes present in the promoter region and the first intron of *p16*, thus upregulating *p16*.⁵

The *p16* promoter guanine cytosine (GC)-rich region comprises no less than five putative GC boxes for Sp transcription factors, including a positive transcription regulatory element for Sp1 binding.⁵

The HMG box-containing protein 1 (HBP1) is a transcription factor in the Ras-Raf-Mek signalling pathway, which has a binding site in the *p16* promoter also containing a binding site for the HBP1, a transcription factor in the Ras-Raf-MEK pathway and this may play a role in the ageing process mediated by *p16*.⁵

In addition, the *p16* promoter contains a binding site for the *INK4a* transcription silence element, a negative regulatory element.⁵

Peroxisome proliferator-activated receptor alpha (PPAR α) inhibits cell cycle progression at the G1/S transition. The binding of PPAR α to the peroxisome proliferator response element (PPRE) region and interaction with Sp1 bound to the *p16* promoter leads to overexpression of *p16* mRNA.⁵

There is also a complex control of *p16*, *p14ARF* and *p15* cluster of genes, which involves several mechanisms. PcG proteins, which include Bmi1, Cbx7, Ring1b or Phc2, are transcriptional repressors that modulate histone changes, resulting in the silencing of transcriptional chromatin, particularly that of groups of genes. Expression of PcG proteins leads to downregulation of *p16*, *p14ARF* and *p15*.⁵

Post-translational regulation

Phosphorylation of *p16* is increased in senescence epithelial cells. Phosphorylated *p16* displays enhanced binding affinity with CDK4/6 compared with unphosphorylated *p16*, leading to G1 arrest in senescence. Different residues of *p16* may be phosphorylated and phosphorylation may eliminate most of *p16* CDK4-inhibitory activity, affecting the core structure or the conformational stability. In addition, any event that affects *p16* phosphorylation may perturbate *p16* activity.

Protein phosphorylation is also strictly linked to the degree of oxidative stress inside cells. Thus, oxidative stress can promote phosphorylation of *p16* phosphorylation, leading to arrest of cell division and early senescence, preventing cells from becoming neoplastic.⁵

Many proteins positively/negatively regulate the *p16*/CDK4 interaction and consequent CDK4-mediated phosphorylation of pRb.⁵

Inhibitor KIP proteins (CDK inhibitor proteins), such as p21, p27 and p57, are able to inhibit the majority of the CDK-cyclin complexes along with some kinases unrelated to CDKs.⁵ The KIP/CDK4/6 complex may dislocate *p16* from CDK4/6 enabling *p16* to be involved in other pathways apart from phosphorylation via pRb CDK4/6 or to be degraded.⁵

GRIM-19 suppression is accomplished by direct interaction with *p16*, thus promoting *p16* inhibition of pRb phosphorylation by CDK4/6. CDK4, *p16* and GRIM-19 form a ternary complex. GRIM-19 promotes the binding of *p16* to CDK4, while the overexpression of cyclin D1 results in loss of the CDK4/*p16*/GRIM-19 complex.⁵

p16 can bind to and suppress NF- κ B, I κ B α , an inhibitor of NF- κ B, which contends with *p16* for CDK4 binding and inhibits phosphorylation of pRb by CDK4.⁵

Gankyrin and *p16* compete for binding to CDK4; however, CDK4 is not inhibited by the binding to Gankyrin, thus resulting in cell cycle progression.⁵

P34^{SEI-1} binds to only CDK4 which seems to oppose *p16* activity, making pRb phosphorylation by CDK4 resilient to *p16* inhibition during late G1 phase.⁵

Tax is a transcription activator encoded by exon 2 of the human T lymphotropic virus 1 genome. This can form a complex with *p16* preventing the inhibition of CDK4/6 by *p16*, resulting in cell cycle progression.⁵

Regulation of *p16* in cancer

Genetic inactivation of *p16* is frequently found in cancer.

Diverse mechanisms such as overexpression of *cyclin D1* (*CCND1*), *gankyrin* (*PSMD10*), *SEI-1* (*SERTAD1*), *CDC6* and NF- κ B can affect the regulation of *p16* function.

The regulation of *p16* in human cancers depends on the effects of the genetic abnormalities of *p16*, activation of oncogenes and changes in related tumour suppressors.

Partial absence of *p16* function secondary to missense mutations of *p16* can be balanced by increased *p16* levels observed in some tumours.

Even if *p16* is preserved, additional molecular changes resulting from overexpression of *CDC6*, *cyclin D1*, *gankyrin* and *SEI-1* may functionally inactivate *p16*, causing cancer progression.

In cancers, *p16* functionality is not inevitably a reflection of the genetic status of *p16* itself. The *p16* status secondary to genetic inactivation and oncogene deregulation may differ during different stages of tumour growth: during the earliest stages, deregulation of *p16* is most likely secondary to activation

Table 1 Cancers and frequency of *p16* inactivation (adapted from Liggett and Sidransky⁷)

Cancer type	% homozygous deletion	% mutation
Oesophagus	20	30
Pancreas	30	30
Non-small cell lung	20	15
Head and neck	50	10
Glioma	60	<5
Bladder	45	<5
Mesothelioma	50	<5
T-ALL	50	<5
Melanoma	25	<5
Ovarian	10	<5
Sarcomas	10	<5

of oncogenes and may occur before the genetic alterations of *p16*.

Finally, oncogene-mediated *p16* deregulation may affect *p16* activity more dramatically than its genetic inactivation.

The overexpression of *p16* in cancers is not well understood. Overexpression of wildtype or mutant *p16* is associated with poor outcomes in several malignancies (neuroblastoma, uterine cervix, ovarian, breast, prostate and oesophageal squamous cell carcinomas). Overexpression of *p16* mutants may be an attempt to restore *p16* functions in some tumours and may be promoted by stress or oncogenic factors but its negative impact on cellular proliferation can be avoided or prevented by alternative molecular events.⁵

Tumours in which *p16* is mutated and use of *p16* immunohistochemistry in diagnostic pathology

Malignancies of almost every type have displayed mutations or molecular alterations of *p16*. Indeed, Liggett and Sidransky suggest that the frequency of *p16* inactivation occurs in about 26% of all malignancies, thus making it second to *p53* in terms of frequency of cancer involvement.⁷ Table 1, adapted from their paper, reflects the range of tumours displaying *p16* aberrations.⁷

The following section highlights the role of *p16* immunohistochemistry and/or fluorescent in-situ hybridisation (FISH) in tumours.

Melanoma

p16 is particularly implicated in familial melanomas but is also involved in sporadic cases. Familial melanoma, which accounts for approximately 5%–10% of all melanomas, arises in the setting of familial atypical multiple mole/melanoma (FAMM).⁷ Germline point mutations in *p16* are seen in FAMM kindreds, and because of this, *p16* is regarded as the 'familial melanoma gene'.

A study of the use of immunohistochemistry for *p16* in Spitzoid lesions was undertaken.⁸ Loss of *p16* expression was not apparent in Spitz nevi (in other words, *p16* immunoreactivity was present/retained), but loss of *p16* was seen in 26% of atypical Spitzoid lesions and 16% of Spitzoid melanomas.⁸ It is interesting to note that desmoplastic melanomas, on the other hand, retain *p16* immunohistochemistry and are *p16* immunopositive (figure 3). Others have advocated combining *p16* immunohistochemistry with Ki-67 and HMB-45 as part of a scoring tool in distinguishing melanoma from benign histological mimicker.⁹

Figure 3 *p16* immunoexpression showing retention (nuclear staining) in a desmoplastic malignant melanoma.

Fatty tumours

The morphological distinction of well-differentiated liposarcoma or atypical lipomatous tumour from benign fat lesions, especially, deep-seated lipomas can be challenging.¹⁰ While there are subtle microscopic clues and location is of paramount importance, sometimes one has to resort to ancillary techniques to resolve a diagnostic dilemma. Immunohistochemistry is now part of the armamentarium in the workup of such lesions with *p16*, CDK4 and MDM2 (including MDM2 amplification via FISH) playing a diagnostic role.^{11 12} *p16* nuclear immunopositivity, in both well-differentiated and dedifferentiated liposarcoma, either alone or in conjunction with CDK4 and MDM2 is of diagnostic help^{11 12} (figure 4). *p16* positivity when coupled with CDK4 positivity is a very sensitive and specific marker for well-differentiated/atypical lipomatous tumour and dedifferentiated liposarcoma.^{11 12} Immunohistochemistry for *p16* in benign fat tumours (lipomas) is negative. An important pitfall to be aware of is nuclear *p16* expression in areas of fat necrosis, especially in deep-seated lipomas.¹³

Mesothelioma

Chromosome 9p alterations are among the most frequent molecular aberrations seen in malignant mesotheliomas.¹⁴ Approximately 30% of malignant mesotheliomas show one of three alterations of the *p16* gene: methylation, deletion or a point

Figure 4 A well-differentiated (atypical) liposarcoma displaying nuclear *p16* immunoreactivity.

mutation.¹⁴ Immunohistochemistry for p16 shows that almost all benign and reactive mesothelial proliferations retain nuclear positivity for p16. Conversely, malignant mesotheliomas demonstrate loss of nuclear staining for p16. However, it is important to recognise that failure to show loss of expression of nuclear p16 does not exclude a diagnosis of malignant mesothelioma. With this in mind, FISH for *p16* has become *de rigueur* part of the work for malignant mesothelioma. It is a more reliable method of assessing p16 status in mesothelial lesions. In a comparison of immunohistochemistry and FISH for *p16*, Chiosea and colleagues showed that the former was less sensitive.¹⁵ In their series, both benign and malignant mesothelial proliferations showed p16 immunopositivity.¹⁵ While benign proliferations more commonly (87% of cases) retained nuclear p16, 60% of malignant lesions also were positive including almost 30% of peritoneal mesotheliomas.¹⁵ In view of this, FISH for *p16* is recommended. It should also be noted that there is no direct correlation protein expression of p16 and homozygous deletions of the gene. FISH for *p16* and BAP1 immunohistochemistry (loss of BAP1 is seen in malignant mesotheliomas) even in effusion cytology specimens is recommended.¹⁶

Anogenital lesions

Uterine cervical and anal squamous epithelia are the site of human papillomavirus (HPV) infection and retinoblastoma (Rb) is inactivated by HPV E7. As a result of this inactivation of Rb, there is no longer inhibition of p16 which then accumulates. This is a reproducible enough phenomenon that p16 protein accumulation as detected by immunohistochemistry is regarded as a reliable surrogate marker for infection by high-risk HPV.¹⁷

In terms of localisation of p16 protein, it should be nuclear or nuclear and cytoplasmic.¹⁸ This was incorporated into the Lower Anogenital Squamous Terminology guidelines that stipulate only diffuse strong nuclear or nuclear and cytoplasmic positivity should be regarded as positive.¹⁹ Cytoplasmic only, focal, weak wispy and scattered single cell positivity is regarded as negative.¹⁹

Normal squamous epithelium is usually negative for p16 (figure 5A). The diffuse strong nuclear or nuclear and cytoplasmic or 'block' positivity covering at least one-third of the epithelial thickness is reflective of high-grade dysplasia (figure 5B). Focal or patchy staining is indicative of a low-grade squamous intraepithelial and maybe associated with low-risk or high-risk HPV genotypes.¹⁹

Figure 5 Normal squamous epithelium from the uterine cervix shows absence of p16 immunoexpression (A), while a high-grade dysplastic lesion is diffuse and strongly p16 positive in the nucleus and cytoplasm (B).

Figure 6 An endocervical glandular carcinoma in situ is strongly p16 positive in a similar fashion to the HPV-driven squamous intraepithelial lesions.

Atrophic squamous epithelium, immature squamous metaplasia and transitional metaplasia may all mimic squamous dysplasia and the use of p16 immunohistochemistry helps resolve the diagnostic dilemma as these conditions are usually p16 negative.¹⁷ Immature squamous metaplasia can be p16 positive (see below).

Mahajan pointed out some important caveats that need to be borne in mind when interpreting p16 immunostaining.¹⁹ One should be aware of p16-negative high-grade dysplastic lesions and correlation with morphology is important; in poorly oriented small biopsies, strong diffuse staining may be hard to evaluate, benign ciliated cells and occasionally immature squamous can display strong p16 positivity and p16 is not useful in separating low-grade squamous intraepithelial lesions from normal and reactive squamous epithelium.^{13 19}

Uterine cervical glandular lesions

Normal uterine endocervical glands are usually p16-negative with weak positivity encountered rarely.¹⁷ Similar to squamous lesions of the cervix that are associated with HPV, a similar relationship with HPV exists in glandular lesions; hence, a similar p16 staining pattern is encountered (figure 6). It is only the endocervical adenocarcinomas that are not associated with high-risk HPV genotypes that are p16-negative. p16 immunohistochemistry can be used effectively in excluding adenocarcinoma in situ from benign look-alikes as the former is diffusely and strongly positive and the mimics are negative or only weakly/patchily positive. In this diagnostic scenario, combining p16 immunohistochemistry with MIB1 and bcl2 is advocated.^{17 20}

p16 immunohistochemistry is also useful in separating endocervical adenocarcinoma from endometrioid carcinomas, especially in biopsy or curettage material.^{17 19} The former shows strong diffuse positivity while endometrial carcinomas are usually negative or focal positivity. Care must be exhibited in small biopsies where the focal positivity of endometrioid carcinoma may be sampled.

Other gynaecological tumours

Vulval intraepithelial neoplasia associated with HPV is similar to cervical squamous epithelial and is p16 positive.¹⁷ p16 is strongly positive in small cell carcinomas, uterine serous and

Figure 7 An oropharyngeal squamous carcinoma (A) showing strong nuclear and cytoplasmic staining in over 70% of the tumour cells (B).

high-grade serous carcinomas of Mullerian origin.¹⁹ Leiomyosarcomas are said to overexpress p16 protein and this may help in separating them from smooth muscle tumours of uncertain malignant potential.¹⁷

Oropharyngeal squamous carcinoma

Once again, p16 is used as a proxy maker for HPV; p16 immunopositivity reflecting an HPV aetiology to the squamous cell carcinoma (figure 7). However, it has been mentioned that there is false-positive p16 immunoreactivity.^{21 22} The reason advanced for the discrepancy the lack of reproducibility regarding percentage of tumour positivity, intensity of staining and cellular distribution of the staining.¹⁹ It is recommended that 70% of tumour cells show strong, diffuse nuclear or nuclear and cytoplasmic p16 staining for the tumour to be labelled as HPV-associated.²³

Take home points

- p16 is the second most common tumour suppressor gene in all cancers.
- p16 is regarded as the familial melanoma gene.
- p16 fluorescent in-situ hybridisation analysis is important in the diagnosis of malignant mesothelioma.
- p16 immunopositivity is seen in well-differentiated and dedifferentiated liposarcoma.
- p16 is a surrogate maker for HPV and is overexpressed in HPV-associated tumours.
- p16 immunopositivity should be strong, diffuse, nuclear or nuclear and cytoplasmic in location.

Handling editor Cheok Soon Lee.

Contributors Both authors contributed equally.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Not required.

Provenance and peer review Commissioned; internally peer reviewed.

REFERENCES

- 1 Serrano M. The tumor suppressor protein p16INK4a. *Exp Cell Res* 1997;237:7–13.
- 2 Komata T, Kanzawa T, Takeuchi H, et al. Antitumour effect of cyclin-dependent kinase inhibitors (p16(INK4A), p18(INK4C), p19(INK4D), p21(WAF1/CIP1) and p27(KIP1)) on malignant glioma cells. *Br J Cancer* 2003;88:1277–80.
- 3 Hannon GJ, Beach D. p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* 1994;371:257–61.
- 4 Robertson KD, Jones PA. Tissue-specific alternative splicing in the human INK4a/ARF cell cycle regulatory locus. *Oncogene* 1999;18:3810–20.
- 5 Li J, Poi MJ, Tsai MD. Regulatory mechanisms of tumor suppressor P16(INK4A) and their relevance to cancer. *Biochemistry* 2011;50:5566–82.
- 6 Lin YC, Diccianni MB, Kim Y, et al. Human p16gamma, a novel transcriptional variant of p16(INK4A), coexpresses with p16(INK4A) in cancer cells and inhibits cell-cycle progression. *Oncogene* 2007;26:7017–27.
- 7 Liggett WH, Sidransky D. Role of the p16 tumor suppressor gene in cancer. *J Clin Oncol* 1998;16:1197–206.
- 8 Harms PW, Hocker TL, Zhao L, et al. Loss of p16 expression and copy number changes of CDKN2A in a spectrum of spitzoid melanocytic lesions. *Hum Pathol* 2016;58:152–60.
- 9 Uguen A, Talagas M, Costa S, et al. A p16-Ki-67-HMB45 immunohistochemistry scoring system as an ancillary diagnostic tool in the diagnosis of melanoma. *Diagn Pathol* 2015;10:195.
- 10 McCarthy AJ, Chetty R. Tumours composed of fat are no longer a simple diagnosis: an overview of fatty tumours with a spindle cell component. *J Clin Pathol* 2018;71:483–92.
- 11 He M, Aisner S, Benevenia J, et al. p16 immunohistochemistry as an alternative marker to distinguish atypical lipomatous tumor from deep-seated lipoma. *Appl Immunohistochem Mol Morphol* 2009;17:51–6.
- 12 Thway K, Flora R, Shah C, et al. Diagnostic utility of p16, CDK4, and MDM2 as an immunohistochemical panel in distinguishing well-differentiated and dedifferentiated liposarcomas from other adipocytic tumors. *Am J Surg Pathol* 2012;36:462–9.
- 13 Ng W, Messiou C, Smith M, et al. p16 Expression in Fat Necrosis: A Potential Diagnostic Pitfall in the Evaluation of Differentiated Adipocytic Neoplasms. *Int J Surg Pathol* 2015;23:544–8.
- 14 Hirao T, Bueno R, Chen CJ, et al. Alterations of the p16(INK4) locus in human malignant mesothelial tumors. *Carcinogenesis* 2002;23:1127–30.
- 15 Chiosea S, Krasinskas A, Cagle PT, et al. Diagnostic importance of 9p21 homozygous deletion in malignant mesotheliomas. *Mod Pathol* 2008;21:742–7.
- 16 Hwang HC, Sheffield BS, Rodriguez S, et al. Utility of BAP1 Immunohistochemistry and p16 (CDKN2A) FISH in the Diagnosis of Malignant Mesothelioma in Effusion Cytology Specimens. *Am J Surg Pathol* 2016;40:120–6.
- 17 O'Neill CJ, McCluggage WG. p16 expression in the female genital tract and its value in diagnosis. *Adv Anat Pathol* 2006;13:8–15.
- 18 Sano T, Oyama T, Kashiwabara K, et al. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. *Am J Pathol* 1998;153:1741–8.
- 19 Mahajan A. Practical issues in the application of p16 immunohistochemistry in diagnostic pathology. *Hum Pathol* 2016;51:64–74.
- 20 Cameron RJ, Maxwell P, Jenkins D, et al. Immunohistochemical staining with MIB1, bcl2 and p16 assists in the distinction of cervical glandular intraepithelial neoplasia from tubo-endometrial metaplasia, endometriosis and microglandular hyperplasia. *Histopathology* 2002;41:313–21.
- 21 Jordan RC, Lingen MW, Perez-Ordóñez B, et al. Validation of methods for oropharyngeal cancer HPV status determination in US cooperative group trials. *Am J Surg Pathol* 2012;36:945–54.
- 22 Lewis JS, Thorstad WL, Chernock RD, et al. p16 positive oropharyngeal squamous cell carcinoma: an entity with a favorable prognosis regardless of tumor HPV status. *Am J Surg Pathol* 2010;34:1088–96.
- 23 Lewis JS, Beadle B, Bishop JA, et al. Human papillomavirus testing in head and neck carcinomas: guideline from the college of American pathologists. *Arch Pathol Lab Med* 2018;142:559–97.
- 24 Byeon JJ, Li J, Ericson K, et al. Tumor suppressor p16INK4A: determination of solution structure and analyses of its interaction with cyclin-dependent kinase 4. *Mol Cell* 1998;1:421–31.