Review

THE *CDKN2A (p16)* GENE AND HUMAN CANCER

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SUMMARY

CDKN2A, the gene encoding the cell-cycle inhibitor $p16^{CDKN2A}$, was first identified in 1994. Since then, somatic mutations have been observed in many cancers and germline alterations have been found in kindreds with familial atypical multiple mole/melanoma (FAMMM), also known as atypical mole syndrome. In this review we tabulate the known mutations in this gene and discuss specific aspects, particularly with respect to germline mutations and cancer predisposition.

INTRODUCTION

CDKN2A—the Gene

CDKN2A has been given different names ($p16^{INK4}$, $p16^{INK4A}$, *CDK4I, MTS1*, and *p16*) by different investigators, but was recently assigned the designation *CDKN2A* (for *c*yclin *d*ependent *k*inase i*n*hibitor *2A*) by the Human Genome Organisation nomenclature committee. The gene is composed of 3 exons, with one alternatively spliced exon (E1-β). It is situated on chromosome 9p21, in a region that shows a high frequency of loss of heterozygosity (LOH) in numerous tumor types (1). The gene itself is mutated (or inactivated in some way) in many types of human cancers. In this sense, *CDKN2A* bears a striking resemblance to the paradigmatic tumor suppressor gene, *p53*. *CDKN2A* may prove to be as important a regulator of cell growth as *p53*.

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An interesting parallel between these two genes can be drawn upon examination of the tumor spectrum observed in families carrying germline mutations. Although both genes are somatically altered in a wide variety of cancers, there is a more specific pattern of malignancy associated with germline mutations. For instance, *CDKN2A* somatic mutations (mainly deletions) are common in glioblastoma (2), but a case of glioblastoma has not yet been reported in a familial atypical multiple mole/melanoma (FAMMM) kindred, where cutaneous malignant melanoma is by far the commonest cancer (3). Similarly, colorectal and ovarian cancer frequently exhibit somatic *p53* mutations (4). However, these cancers are rarely seen as part of the familial Li-Fraumeni syndrome, which typically features soft tissue sarcomas, leukemia, and brain cancers in children, and breast cancer in young women (5).

$p16^{CDKN2A}$—the Protein

CDKN2A encodes a 156 amino acid, 16kD cell-cycle inhibitor protein, which normally blocks

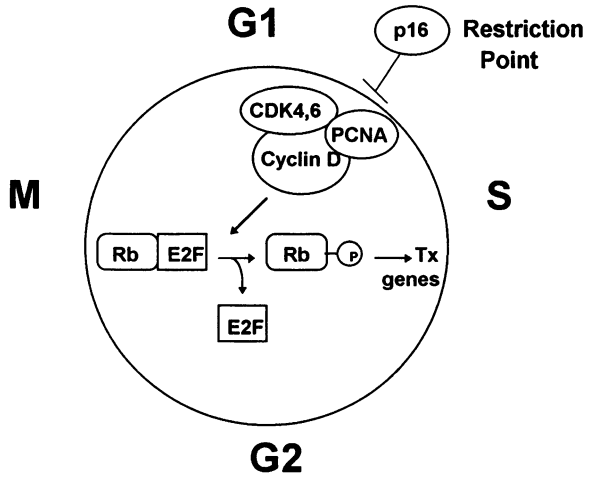

FIG. 1. Diagrammatic representation of the involvement of (p16) in cell cycle regulation

Abbreviations are as follows: CDK, cyclin-dependent kinase; PCNA, proliferating cell nuclear antigen; Rb, retinoblastoma gene product; E2F, transcription factor; P, phosphate; Tx genes, gene transcription; p16, *CDKN2A* gene product.

abnormal cell growth and proliferation by binding to complexes of cyclin-dependent kinases (CDK) 4 and 6, and cyclin D. This binding inhibits the kinase activity of the enzyme, which arrests the cell cycle in the G1 phase (Fig. 1). Mutant $p16^{CDKN2A}$ is unable to form stable complexes with the enzyme and, therefore, does not effectively inhibit progression of cells through inappropriate mitotic divisions. The importance of this protein as a cell-cycle regulator is demonstrated by the wide array of tumor types in which mutations of *CDKN2A* have been observed.

IDENTIFICATION OF *CDKN2A* AS A POSSIBLE TUMOR SUPPRESSOR GENE

Molecular cytogenetic studies revealed frequent heterozygous and homozygous deletions of the chromosome 9p21-p22 region in melanoma and other cell lines (6,7). Linkage to markers from 9p13-p22 was reported in melanoma kindreds from the United States (8,9), Australia (10), Holland (11), and the United Kingdom (12). *CDKN2A* mutations were subsequently reported in several families within each cohort. However, there appear to be some families that are linked to 9p21 in which mutations in *CDKN2A* or the closely related adjacent gene, *CDKN2B (p15)*, have not been found.

Homozygous deletions and intragenic mutations in *CDKN2A* are often observed in human cell lines. Initially, there was some controversy over the significance of these alterations, since they were detected far less frequently in corresponding primary tumors showing LOH at 9p21 (1,13–15). This discrepancy suggested that another tumor suppressor gene is the target of LOH in the region or simply that *CDKN2A* is important for the maintenance of viability in cell culture. The issue has been clarified to some extent by two recent discoveries that suggest that LOH at 9p21 may be accounted for by previously undetected mutations in *CDKN2A*. First, microdeletions (spanning less than 200 kilobases and encompassing *CDKN2A*) are present; these are, however, only detectable using an array of microsatellites close to the gene and have been confirmed by fluorescent in situ hybridization (FISH) (16). Secondly, *CDKN2A* can be inactivated by methylation of the CpG island 5′ of the coding region (17–19). The involvement of several mechanisms in *CDKN2A* inactivation has been shown in a comprehensive analysis of 29 primary head and neck squamous cell carcinomas. By immunohistochemistry, 24 of the cancers showed absence of nuclear staining. Of these 24 cancers, 16 had homozygous deletions of *CDKN2A*, 5 had CpG methylation, 1 had a genomic rearrangement, and another had a frameshift mutation in exon 1 (20).

GERMLINE MUTATIONS

Germline mutations in *CDKN2A* reported to date are primarily point mutations (Fig. 2). They are observed in FAMMM families but also in cancer-prone kindreds which do not fit the criteria for FAMMM.

Familial Atypical Multiple Mole/Melanoma (FAMMM)

Germline mutations in *CDKN2A* have been reported in FAMMM pedigrees that show linkage to chromosome 9p. Hussussian et al. described eight *CDKN2A* germline substitutions observed in 13 of 18 American FAMMM kindreds (21). Of these, six are probably disease-related mutations since they were identified in 33 out of 36 melanoma cases in nine families. The remaining two

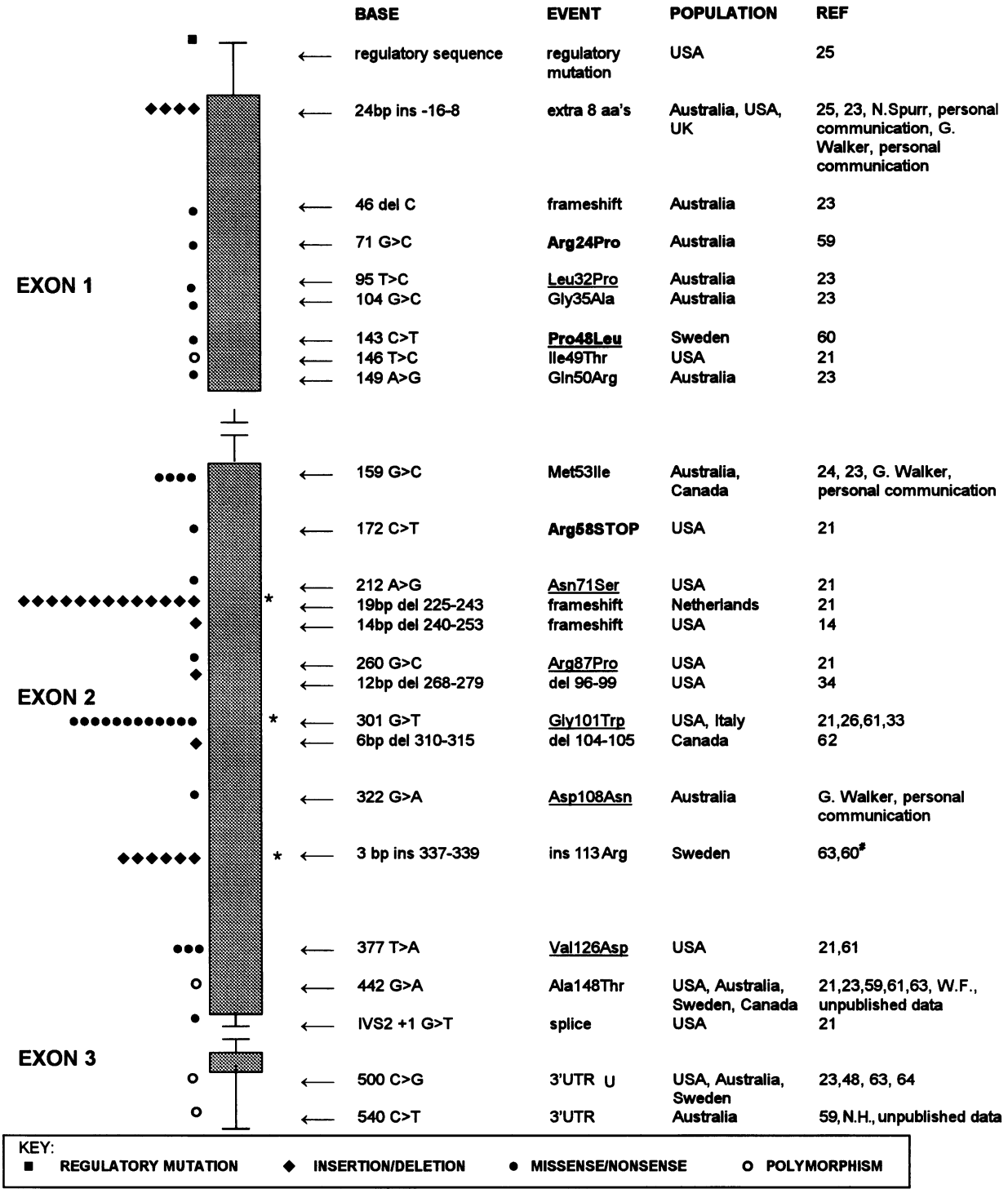

FIG. 2. Germline mutations and polymorphisms in *CDKN2A* identified to date (July 1996) and their relative frequencies

Although the *CDKN2A* germline variants are presented here in melanoma kindreds, this term does not apply to all of the families listed here: the Met53Ile (23), Gly101Trp (33), and 12 bp del 268-279 (34) have all been reported to occur in kindreds with only one case of melanoma. Each symbol to the left of the diagram represents one family in which the corresponding mutation has been found. Underlined mutations occur in consensus ankyrin domain amino acids. Mutations in bold are also seen somatically in primary tumors and/or cell lines. Mutations accompanied by asterisks have some kindred sharing a common founder. #In the original reference, this mutation was reported as Ins111Arg. This is probably the same mutation as the Ins113Arg reported by Borg et al. (63).

were detected in normal controls and are thought to be nondeleterious polymorphisms.

A 19 base pair germline deletion in *CDKN2A* was detected in 13 of 15 Dutch FAMMM kindreds (22). This deletion is a founder mutation; the 13 families all originate from the same geographic region and share a common haplotype. *CDKN2A* mutations were not found in two other Dutch melanoma families. Two individuals from one of the families carrying the 19 bp deletion were homozygous for the mutation. One of these individuals had three mildly atypical nevi and died at the age of 54 from adenocarcinoma originating from an unknown primary site and the other was diagnosed with melanoma in situ at age 15. The fact that these two homozygous individuals did not have more severe phenotypes than their heterozygous relatives suggests that there may be redundancy that compensates for the lack of $p16^{CDKN2A}$ activity.

The Met53Ile mutation (Fig. 2) could also be a founder mutation, since the antecedents of the three Australian melanoma kindreds reported with this mutation all originally lived in Scotland (23) and another report of a family with this mutation identified the place of origin as the west of Scotland (24), although this family does not fit the FAMMM criteria. Haplotype analyses are now underway but have yet to be completed.

CDKN2A, FAMMM, and Pancreatic Cancer

Pancreatic adenocarcinoma is probably the second commonest cancer in FAMMM families. The observed/expected ratio for the frequency of pancreatic cancer among 200 individuals from nine FAMMM families was 13.4 ($P < 0.001$) (3). In several chromosome 9p-linked FAMMM families, a mutation in *CDKN2A* was found to cosegregate with both melanoma and pancreatic adenocarcinoma (21,22,25). However, this risk may be limited to a subset of families. In families where the *CDKN2A* mutation impaired the function of the corresponding protein (p16M) in vitro, the risk of pancreatic cancer was increased 13-fold (standardized incidence ratio: 13.1, 95% CI, 1.5–47.4), whereas no cases of pancreatic cancer were found in families with *CDKN2A* mutations that did not affect the function of the protein (p16W) in the assay used by this group (25).

The study of Ciotti et al. supports the association of p16M mutations and pancreatic cancer risk in FAMMM families (26). They detected a Gly101Trp missense mutation in seven Italian melanoma-prone kindreds (presumably derived from a common founder) having a combined total of three pancreatic cancers. This mutation affected the function of the $p16^{CDKN2A}$ protein in vitro, and was associated with increased cancer risk (27). Seven p16W melanoma-prone kindreds, on the other hand, had a total of 18 cases of melanoma, 4 cases of dysplastic naevi, and 9 cancers at other sites, but no cases of pancreatic cancer (26).

However, this excess of pancreatic cancer in FAMMM families may be attributed, in part, to ascertainment bias. Investigations of the tumor spectrum of the FAMMM syndrome revealed no excess of pancreatic tumors in a total of 15 FAMMM kindreds and 370 individuals diagnosed with melanoma (28–30). The results from Australian kindreds (N.H., unpublished data) support this lack of excess of pancreatic cancer in FAMMM pedigrees: they found one case of pancreatic cancer in 11 families with p16M mutations (0.4 cases expected). This difference is not significant (95% CI, 0.49–24.56). Overall, there were slightly more cancers of all types among these families than among those with p16W *CDKN2A* mutations, but again, this difference was not significant.

It may be that the risk of pancreatic cancer is related to the position or type of *CDKN2A* mutation; in von Hippel Lindau disease kindreds, the risk of pheochromocytoma appears to be greatest in those with missense mutations in *VHL* (31), and in hereditary breast cancer, the risk of ovarian cancer may depend upon the position of the mutation along the *BRCA1* gene (32).

CDKN2A Mutations in Melanoma Families Apparently Not Linked to 9p

Because of the high phenocopy rate, mutations have also been reported in families inconclusive for linkage to 9p (21,23). Some cases of melanoma observed in these families appear to be sporadic in origin and, in contrast to the definitively 9p-linked families, *CDKN2A* mutants in these unlinked kindreds did not always segregate with the melanomas.

Non-FAMMM Kindreds with *CDKN2A* Mutations

CDKN2A mutations are not restricted to the FAMMM syndrome. Interestingly, however, there are no reported mutations in families with multiple cancers that do not include melanoma.

A family in which pancreatic cancer was the predominant cancer has been reported (33). A p16M missense mutation (Gly101Trp) was found in all affected family members, but only one individual was affected with melanoma (followed by pancreatic cancer). Sun et al. detected a Met53Ile *CDKN2A* mutation in a non-FAMMM family with an excessive number of cancers. One member of the family developed two cutaneous malignant melanomas during pregnancy, but no other individuals have had melanomas or dysplastic nevi. A p16M (deletion of amino acids 96–99) mutation was reported in a family with melanoma, nonsmall cell lung cancer, and squamous cell carcinoma of the head and neck (34). It appears that *CDKN2A* is highly penetrant for cutaneous malignant melanoma, and the absence of this cancer in a pedigree with multiple cases of other cancers is likely to imply that a *CDKN2A* mutation is not present in that family (W.F., unpublished data).

SOMATIC MUTATIONS

CDKN2A is frequently homozygously deleted in cell lines derived from a number of tumor types (13,16,35). In melanoma cell lines where one copy of the gene is absent, the remaining copy is frequently mutated (7). The majority of point mutations observed are nonsense, missense, or frameshift mutations. Details of the coding effect of *CDKN2A* mutations can be found in a recent review by Pollock et al. (36). We have built upon the databases compiled by Pollock et al. (36) and Smith-Sorensen and Hovig (37) of *CDKN2A* somatic mutations in cell lines and in primary tumors, and present updated lists (to July 1996) in Tables 1 and 2, respectively.

It is interesting to note that alterations have been reported to occur in at least 70% of the possible 156 codons making up this small gene (deletions affecting more than one codon were counted only once). Moreover, only 11% of the germline mutations identified to date (Fig. 2) have been seen somatically (in either primary tumors or cell lines). There are several possible explanations for the latter phenomenon: (*1*) ascertainment bias—novel germline mutations may be detected in kindreds displaying different phenotypes than the families tested so far; (*2*) deleterious mutations—certain *CDKN2A* germline mutations may not be compatible with life; (*3*) somatic mutations reflect mutagenesis by a different carcinogen (i.e., ultraviolet radiation induces characteristic transitions and tandem base changes); and, (*4*) the lack of concordance between somatic and germline mutations may be attributed to chance.

Homozygous Deletions in Cell Lines

There have been numerous reports of homozygous deletion of the 9p21 region in cell lines derived from a wide variety of human tumors (7,35). *CDKN2A* has often (but not always) proven to be the target of these deletions. If a gene is the sole target of homozygous deletion, one would expect to find intragenic mutations in the DNA of cell lines without homozygous deletions. This criterion is not always met in the case of *CDKN2A*, however, which suggests the presence of another gene in the region that is responsible for the deletions observed; the neighboring *CDKN2B* gene is an obvious candidate. Microdeletions, aberrant CpG island methylation, or other regulatory mutations in *CDKN2A* not detected by the assays used by these investigators, may account for part of the reported absence of homozygous deletions (see above).

Cell lines with deletions of *CDKN2A* alone, *CDKN2B* alone, and with codeletion of the two genes have all been observed (2,13,38–45). *CDKN2A* is usually but not always included in the smallest region of homozygous deletion (2,38,39,46). This result can be interpreted in various ways: (*1*) the target gene is *CDKN2A* in some cases and *CDKN2B* in others; (*2*) both genes together are the target of the deletions; or (*3*) other tumor suppressor genes are present in the region. Jen et al. (38) favor the "double target" hypothesis because the protein products of the two genes appear to have analogous biochemical activities and the region of homozygous deletion most often contains both genes. A more definitive explanation awaits further functional analyses of the genes, especially *CDKN2B*.

Common Tumors with a Low Frequency of *CDKN2A* Mutations

COLON CARCINOMA. *CDKN2A* mutations are rarely observed in colon cancer (7,13,16,38). Interestingly, aberrant 5′ CpG island methylation associated with loss of transcription of *CDKN2A* occurs frequently in both colon cancer cell lines and primary colon tumors (18). Of all the cell lines tested, those derived from colon primaries showed the highest frequency of de novo meth-

ylation (92%). CpG island methylation accompanied by transcriptional silencing of *CDKN2A* was also observed in the normal colonic mucosa of individuals with and without cancer (19). It is uncertain whether this uncommon situation represents a precancerous state or normal cell function. In general, these data point to hypermethylation as an alternative mechanism of *CDKN2A* inactivation in colon cancer.

BREAST CANCER. *CDKN2A* is homozygously deleted in 60–65% of primary breast tumors and cell lines (7,16), however point mutations in this malignancy are rare (Tables 1 and 2). Of 24 primary breast carcinomas analyzed by Brenner et al., 58% showed LOH or allelic imbalance at 9p21-22, but only 1 of 21 had an intragenic mutation (47). Xu et al. screened 37 primary breast carcinomas and 5 cell lines for alterations in *CDKN2A* by single-strand conformation analysis (SSCA) (48). No mutations were found in any of the tumor samples but the gene was homozygously deleted in 2 of the 5 cell lines. Herman et al. reported CpG island methylation in 33% of breast cancer cell lines (18). Thus, it would appear that the primary mechanisms of *CDKN2A* inactivation in breast cancer are homozygous deletion and hypermethylation, but not point mutation.

OVARIAN CARCINOMA. LOH at 9p21 has been observed in 29–48% of ovarian tumors and cell lines (7,49); however, no tumor-specific *CDKN2A* mutations were detected in a total of 78 primary ovarian tumors analyzed by Campbell et al. (49) and Hatta et al. (50), and homozygous deletions were seen in only 2 tumors. It is not clear whether the high rate of 9p allelic loss in this malignancy is due to mechanisms of *CDKN2A* inactivation other than deletions and point mutations, or whether a different tumor suppressor gene is the target of LOH. There is an interesting parallel on chromosome 18q, where LOH is extremely frequent in colon cancer. At first, the gene *DCC* (51) was thought to be the sole target of the LOH, but it is now apparent that *DPC4* and another related gene, *JV-18-1*, may also be mutated or otherwise inactivated in colorectal cancers (52,53). Similarly, perhaps other 9p21 cancer genes await identification.

The frequency of intragenic mutations in *CDKN2A* is relatively low in uncultured tumors. However, since there are other ways of abrogating $p16^{CDKN2A}$ function, the frequency of inactivation of *CDKN2A* in a given tumor type may in fact be higher than might be predicted from sequence analysis only. If this is the case, overall, *CDKN2A* may be somatically altered at a similar frequency to *p53*.

CDKN2A Mutations, Expression Levels, and Survival

Loss of $p16^{CDKN2A}$ expression may be related to invasiveness or metastatic potential rather than to tumor initiation. This is supported by the study of Reed et al. (54), who performed immunohistochemical analysis of $p16^{CDKN2A}$ expression on 103 melanocytic lesions ranging from atypical nevi to metastatic melanomas. $p16^{CDKN2A}$ expression was detected in 100% of atypical nevi and melanomas in situ and in 91% of primary invasive melanomas, but in only 56% of metastatic melanomas.

Nearly 80% of pancreatic cell lines or xenografts (55) and 37% of primary pancreatic adenocarcinomas (56) have *CDKN2A* mutations or deletions. The status of *CDKN2A* may be related to the prognosis of pancreatic cancer patients: mean survival was 13.5 months longer for individuals with *CDKN2A* mutation-negative tumors compared with those having mutation-positive tumors ($p = 0.017$) (57). It is interesting that LOH at 9p21 in breast cancers, on the other hand, may not confer an adverse prognosis (58). Thus it may be that the poorer survival in pancreatic carcinomas with *CDKN2A* mutations is either because *CDKN2A* mutations have a tumor-specific effect, or because in breast cancer, another gene is the target for the LOH and this gene does not have an adverse effect on survival.

CONCLUSIONS

Over the last 2 years, *CDKN2A* has been the subject of intensive research. It clearly plays a central role in the development of both hereditary and sporadic forms of melanoma. The gene is frequently altered in tumors of many different types, although its importance in most of these cancers can only be inferred. Future reviews of *CDKN2A* will hopefully focus on attempts to further dissect its function and to rectify the defects in vivo.

ACKNOWLEDGMENTS

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TABLE 1. *CDKN2A* Somatic mutations identified to date (July 1996) in cell lines

Base	Event/Codon	Cell Line	Reference
Exon 1			
25 ins TG	9	Pancr adeno	44
35 C>A	Ser12STOP	NSCLC	41
55 ins CGCGCAC	19	Ductal pancr	42
58 ins ACGGCC	20	Pancr adeno	36
58 G>C	Ala20Pro	Melanoma, lung	36
63 del 23 bp	21	Liver	36
? ins 1 bp	23	Colon	36
85 del 18 bp	29	Pancr adeno	42
101 del CGG	34	Ductal pancr	42
104 G>A	Gly35Glu	Melanoma	36
106 G>A	Ala36Thr	Pancr adeno	44
128 del GT	43	Melanoma	36
131 del 33 bp	44	Lung mucoepidermoid	36
134 del G	45	NSCLC SCC	36
142 CC>TT	Pro48Leu	Melanoma	36
143 C>T	Pro48Leu	Melanoma	36
148 C>T	Gln50STOP	Melanoma	36
int1-2 A>C	splice	Chondrosarcoma, NPC	36
int1-2 A>G	splice	Mesothelioma	36
int1-1 G>T	splice	HNSCC	36
int1-1 G>A	splice	NPC	36
Exon 2			
155 T>A	Met52Lys	Ductal pancr	42
156 G>C	Met52Ile	Breast	65
161 del 14 bp	54	Oral SCC	36
167 ins 1 bp	56	Melanoma	36
171 C>A	Ala57Ala	Melanoma	36
171 CC>TT	Arg58STOP	Melanoma	36
172 C>T	Arg58STOP	Bladder, melanoma	36
172 del 8 bp	58	Melanoma	36
174 del 16 bp	58	Oral SCC	36
180 del 29 bp	60	T-ALL	66
180 ins 1 bp	60	Melanoma	36
181 G>T	Glu61STOP	Melanoma	36
182 A>G	Glu61Gly	Pancr adeno	44
183 G>C	Glu61Asp	Oral SCC	67
184 C>G	Leu62Val	Oral SCC	67
199 ins 1 bp	67	Melanoma	36
204 del 5 bp	68	Thyroid	68
205 G>T	Glu69STOP	Melanoma, NSCLC	36,41
206 A>T	Glu69Val	NSCLC SCC	36
207 G>C	Glu69Asp	Oral SCC	67
208 C>G	Pro70Ala	Oral SCC	67

TABLE 1. ***(Continued)***

Base	Event/Codon	Cell Line	Reference
216 C>A	Cys72STOP	NSCLC	41
220 G>A	Asp74Asn	Bladder	36
231 del TCTC	77	Oral SCC	36
233[a] del TC	78	Pancr adeno, bladder, oral SCC	36,37
237CC>TT	Arg80STOP	Melanoma, liposarcoma	36,40
238 C>T	Arg80STOP	Melanoma, myeloid leukemia, T-ALL, Thyroid, Oral SCC	36,67–69
242 C>T	Pro81Leu	Melanoma	36
247 C>T	His83Tyr	Melanoma, NSCLC	36,70
250 G>T	Asp84Tyr	Prostate	36
261 GG>AA	Glu88Lys	Melanoma	36
262 G>A	Glu88Lys	Melanoma	36
262 G>T	Glu88STOP	Melanoma	36
264 GG>AA	Gly89Ser	Melanoma	36
290	5bp del 97	Melanoma	36
295	3bp del 99	Melanoma	36
296 GG>CA	Arg99Pro	Melanoma	36
? G>A	101	Leukemia	71
320 G>A	Arg107His	Leukemia	71
322 G>C	Asp108His	Bladder	36
329 G>A	Trp110STOP	Melanoma	36
330 G>A	Trp110STOP	Melanoma, ovary	36
335 G>C	Arg112Pro	Melanoma	36
341 C>T	Pro114Leu	Melanoma, fibrosarcoma	36
346 G>T	Asp116Tyr	Melanoma	40
358 G>T	Glu120STOP	Oral SCC	67
? del	122	Leukemia	71
? G>C	122	Leukemia	71
369 T>A	His123Gln	NSCLC	41
378 C>T	Val126Val	Melanoma	36,72
386 A>G	Tyr129Cys	Pancr adeno	44
425 A>G	His142Arg	Melanoma	36
int2 +1 G>T	splice	NSCLC	36
int2 +2 T>C	splice	Ductal pancr	55

Underlined mutations appear in both cell lines and primary tumors. Question marks indicate information not specified in the original sources. The following abbreviations appear in the tables: pancr, pancreas; adeno, adenocarcinoma; SCC, squamous cell carcinoma; NSCLC, non-small cell lung cancer; NPC, nasopharyngeal carcinoma; HNSCC, head and neck squamous cell carcinoma (HNC, head and neck cancer); T-ALL, T-lymphocyte acute lymphoblastic leukemia. Since we have built upon the databases of *CDKN2A* somatic mutations compiled by Pollock et al. (36) and Smith-Sorensen and Hovig (37), we do not quote the primary sources for most of the mutations reported in these two publications. Please see these reviews for the original references.

[a]Because of the ambiguity involved in assigning nucleotide positions to certain deletions, this deletion is numbered from where the wild-type sequence first changes.

TABLE 2. *CDKN2A* somatic mutations identified to date (July 1996) in primary tumors

Base	Event/Codon	Tumor	Reference
Exon 1			
?-17 del 24 bp	1–3	Prostate	36
? ins C	4	Melanoma	73
15 del 37 bp	5	Pancr adeno[a]	36
23 del GCATGGA /insTCCCGG	8	ALL	74
27 G>A	Met9Ile	Hilar bile duct	36
33 del 35 bp	11	B-NHL	75
42 C>G	Asp14Glu	Gall bladder	36
47 del 4 bp	16	Pancr adeno[a], esoph SCC, glioblastoma	55,76,77
47 T>C	Leu16Pro	Hilar bile duct	36
52 del 32 bp	18	Pancr adeno	56
57 del C	19	NSCLC	78
58 G>T	Ala20Ser	Gall bladder	36
59 C>A	Ala20Glu	NSCLC	70
68 del G	23	NSCLC	78
68 G>A	Gly23Asp	Pancr[a]	36
71 G>C	Arg24Pro	Sarcoma	45
74 T>C	Val25Ala	Prostate	79
78 G>C	Glu26Asp	Gall bladder	36
? del 21 bp	29	Pancr adeno	56
88 G>C	Ala30Pro	Esoph SCC	36
97 G>T	Glu33STOP	HNSCC	36
99 G>T	Glu33Asp	Hilar bile duct	36
109 C>T	Leu37Leu	Melanoma	80
124 A>G	Asn42Asp	Ductal pancr	42
132 C>A	Tyr44STOP	NSCLC	36
143 C>T	Pro48Leu	HNSCC	36
146 T>G	Ile49Ser	Hilar bile duct	36
148 C>T	Gln50STOP	Esoph SCC	76
150 GG>CC	Gln50 His/ Val51Leu	Melanoma	81
int1-1 G>T	splice	NSCLC SCC	82
int 1-8 del 27 bp	splice	CLL	83
int 1-9 del 61 bp	splice	Bladder	36
int 1-2 A>T	splice	Bladder	36
Exon 2			
151 G>A	Val51Ile	Ductal pancr	42
152 T>A	Val51Asp	Pancr adeno	36
155 T>A	Met52Lys	Breast	36
157 del A	53	Bladder	36
158 T>C	Met53Thr	CLL	83
160 del A	54	Pancr adeno	56
164 del G	55	Esoph SCC	36

TABLE 2. (*Continued*)

Base	Event/Codon	Tumor	Reference
165 ins 1 bp	55	Endomet	36
166 del AG	56	NSCLC SCC	36
169 del 13	57	B-NHL	75
171 del CC	57	Ductal pancr	42
170 C>T	Ala57Val	ALL	36
172 C>T	Arg58STOP	Esoph SCC, bladder, NSCLC SCC, pancr adeno	56,82
174 ins 7 bp	58	T-ALL	66
176 ins G	59	NSCLC adeno	36
181 G>T	Glu61STOP	Esoph SCC, HNSCC	36
192 del GCT	64	NSCLC SCC	36
194 del 50 bp	65	Esoph SCC	36
196 del 1 bp CAC>TA	His66STOP	Melanoma	73
196 C>T	His66Tyr	NSCLC SCC	36
202 G>A	Ala68Thr	Esoph SCC	36
205 G>T	Glu69STOP	NSCLC	41
205 G>A	Glu69Lys	Bladder	37
213 AAC>AAGGTCG	71	T-ALL	66
214 T>G	Cys72Gly	Esoph SCC	36
216 del C	72	NSCLC large cell	82
216 C>A	Cys72STOP	NSCLC	41
217 G>A	Ala73Thr	Glioblastoma	84
220 G>A	Asp74Asn	Esoph SCC	36
221 A>T	Asp74Val	Hepatic bile duct	36
224 C>T	Pro75Leu	Breast	85
226 G>A	Ala76Thr	Esoph SCC	36
227 C>T	Ala76Val	Glioblastoma	84
233# del TC	78	T-ALL	86
238 C>T	Arg80STOP	Esoph SCC, NSCLC SCC, bladder, T-ALL, pancr[a], oral SCC	36,56,67,86
239 del G	80	Esoph SCC, B-NHL	36,75
239 G>T	Arg80Leu	HNSCC	36
242 C>T	Pro81Leu	Melanoma, thyroid	36,87
243 ins 19 bp	82	Pancr adeno	56
247 C>A	His83Asn	NSCLC SCC	36
247 C>T	His83Tyr	HNSCC, pancr[a], breast	36
250 G>A	Asp84Asn	Esoph SCC, NSCLC adeno, HNSCC	36
250 G>C	Asp84His	NSCLC adeno	36
250 G>T	Asp84Tyr	NSCLC SCC	36
252 C>A	Asp84Glu	Bladder	37
253 G>A	Ala85Thr	Glioblastoma	84
257 ins G	86	NSCLC large cell	36
262 G>T	Glu88STOP	NSCLC SCC, melanoma	81,82
264 G>T	Glu88Asp	Gall bladder	36
266 del GC	89	HCC	88

TABLE 2. (*Continued*)

Base	Event/Codon	Tumor	Reference
271# del C	91	Pancr adeno[a], bladder	36,89
274 del G	92	NSCLC SCC	36
277 A>G	Thr93Ala	NSCLC adeno	36
278 C>G	Thr93Arg	Glioma	36
284 T>C	Val95Ala	NSCLC adeno	36
292 C>T	His98Tyr	Glioblastoma	84
293 AC>CT	His98Pro	Melanoma	36
293 A>G	His98Arg	CLL	83
294 C>A	His98Leu	Melanoma	36
296 G>A	Arg99Gln	NSCLC adeno	36
298 GC>CT	Ala100Leu	Melanoma	36
305 C>T	Ala102Val	Glioblastoma	84
307 del CG/ins A	103	NSCLC SCC	82
310 del C	104	Esoph SCC	36
313 del G	105	Esoph SCC	36
314 del 20 bp	105	Pancr adeno[a]	36
316 G>A	Val106Met	Glioblastoma	84
319 C>T	Arg107Cys	Glioblastoma	84
322 G>T	Asp108Tyr	HNSCC, NSCLC SCC	82
329 G>A	Trp110STOP	Melanoma	36
330 G>A	Trp110STOP	Melanoma, pancr[a], glioma, T-ALL	36,90
332 G>A	Gly111Asp	Bladder	89
334 C>G	Arg112 Gly	Melanoma	73
340 C>T	Pro114Ser	Esoph SCC	36
341 C>T	Pro114Leu	Astrocytoma	36
347 A>T	Asp116Val	Prostate, CLL	79,83
350 del T	117	Bladder	89
352-440 88 bp del	118	Glioma	84
355 G>C	Glu119Gln	Gall bladder	36
358 G>T	Glu120STOP	Esoph adeno, NSCLC	36
358 G>A	Glu120Lys	NSCLC adeno, SCC	36
359 A>C	Glu120Ala	NSCLC adeno	36
364 G>A	Gly122Ser	Ampullary	36
365 del G	122	NSCLC SCC	36
369 T>A	His123Gln	CLL	36
371 G>A	Arg124His	Esoph SCC	36
374 del A	125	T-ALL	86
375 T>C	Asp125Asp	Bile duct	36
378 C>T	Val126Val	Bladder	72
379 G>T	Ala127Ser	Bladder	91
380 C>T	Ala127Val	Glioblastoma	84
382 C>T	Arg128Trp	Glioblastoma	84
385 del 23 bp	129	Pancr adeno	56
394 G>C	Ala132Pro	NSCLC adeno	36
401 C>T	Ala134Val	NSCLC adeno	36
405 G>A	Gly135Gly	Gall bladder, glioma, NSCLC, stomach, T-ALL	41,66,92,93

TABLE 2. *(Continued)*

Base	Event/Codon	Tumor	Reference
406 del GG	136	Esoph SCC	36
407 G>A	Gly136Asp	Glioblastoma	84
424 C>T	His142Tyr	NSCLC adeno	36
430 del C	144	Esoph SCC	36
430 C>T	Arg144Cys	Esoph SCC	36
449 G>T	Gly150Val	NSCLC SCC	36
451 C>T	Pro151Ser	Melanoma	80
int2+1 G>T	splice	NSCLC	41
int2+2 T>C	splice	Pancr[a]	36

Underlined mutations appear in both cell lines and primary tumors. Question marks indicate information not specified in the original sources. The following abbreviations appear in the tables: pancr, pancreas; esoph, esophagus; adeno, adenocarcinoma; SCC, squamous cell carcinoma; ALL, acute lymphoblastic leukemia (T-ALL, T-lymphocyte ALL); CLL, chronic lymphoblastic leukemia; NSCLC, non-small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; B-NHL, B-lymphocyte non-Hodgkin's lymphoma; HCC, hepatocellular carcinoma. Since we have built upon the databases of *CDKN2A* somatic mutations compiled by Pollock et al. (36) and Smith-Sorensen and Hovig (37), we do not quote primary sources for most of the mutations reported in these two publications. Please see these reviews for the original references.

[a]Xenograft.

[b]Because of the ambiguity involved in assigning nucleotide positions to certain deletions, these deletions are numbered from where the wild-type sequence first changes.

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