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Review

Updates and Critical Evaluation on Novel Biomarkers for the Malignant Progression of Intraductal Papillary Mucinous Neoplasms of the Pancreas

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Abstract. *Intraductal papillary mucinous neoplasms (IPMNs) are presumed to evolve from low-grade dysplasia to high-grade dysplasia to invasive carcinoma. Resection of lesions before the development of pancreatic cancer may prevent the development of an incurable process as, once IPMNs progress to invasive cancer, the prognosis may be as poor as resected conventional pancreatic ductal adenocarcinoma. Resection of IPMNs, particularly in the setting of high-grade dysplasia, is presumed to provide a survival benefit. IPMNs also present many challenges as the identification of high-grade dysplasia and early invasive carcinoma and the timing and frequency of malignant progression are not yet established. The limited predictive accuracy presents a challenge as pancreatic resection is associated with a risk of substantial morbidity and mortality; 20-30% and 2-4%, respectively. Diagnostic armamentarium contains pancreas-protocol computed tomography (CT) scan, gadolinium-enhanced magnetic resonance imaging (MRI) with or without magnetic resonance cholangiopancreatography (MRCP) and endoscopic ultrasound (EUS). The most promising method is endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) as this technique allows analysis of cyst fluid using biomarkers. Until now, in clinical practice, we utilize two biomarkers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9); however, DNA analysis of pancreatic cystic fluid and genomic analysis could offer new tools to the diagnosis and administration of IPMNs. Novel genomic and serum biomarkers could play an important future role to identify those individuals who will benefit from an early operation and those who will benefit from watchful waiting approach. More prospective studies are needed.*

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Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas represent both an opportunity and a challenge. They represent an opportunity as these lesions are the only radiographically identifiable precursors of pancreatic cancer. These cystic lesions are presumed to evolve from low-grade dysplasia to high-grade dysplasia to invasive carcinoma (1, 2). Resection of lesions before the development of pancreatic cancer may prevent the development of an incurable process as, once IPMNs progress to invasive cancer, the prognosis may be as poor as resected conventional pancreatic ductal adenocarcinoma (3-5). Resection of IPMNs, particularly in the setting of high-grade dysplasia, is presumed to provide a survival benefit. IPMNs also present many challenges as the identification of high-grade dysplasia and early invasive carcinoma and the timing and frequency of malignant progression are not yet established (6, 7).

Currently, the most accurate test for prediction of high-grade dysplasia or invasive disease is dilation of the main pancreatic duct on preoperative imaging (main duct IPMN (MD-IPMN)). Patients who undergo resection for MD-IPMN have an approximately 60% chance of harboring high-grade dysplasia or invasive disease at the time of resection. This high-risk disease is present in approximately 20-25% of patients who undergo resection in the absence of a dilated pancreatic duct (branch duct IPMN (BD-IPMN)) (8). This limited predictive accuracy presents a challenge as pancreatic resection continues to be associated with a risk of substantial morbidity and mortality. In high-volume centers performing pancreaticoduodenectomy, the reported major morbidity rates are approximately 20% to 30% and mortality rates approximately 2% to 4% (9, 10).

The aim of this review is to demonstrate and critically evaluate the evolution and the recent updates on the biomarkers related to malignant progression of pancreatic IPMN.

Current Diagnostic Approach

In general, the diagnosis in IPMNs is associated with the detection of several characteristics of the cysts. These characteristics are related to the prediction of malignancy, MD lesions' involvement and the detection of mural nodule. Concerning the mural nodule, according to reports, the size threshold is 5mm (11, 12). It is well-known that, theoretically, the modalities of choice are either pancreas-protocol computed tomography (CT) scan or gadolinium-enhanced magnetic resonance imaging (MRI) with magnetic resonance cholangio-pancreatography (MRCP). However, in practice, MRI is the preferable imaging method as it succeeds higher contrast resolution in outlining the MD involvement and finding mural nodule. Also, it has the benefit that patients who should be long-term checked avoid repeated radiation exposure (13).

Another imaging method that presents sublime sensitivity and can find mural nodules within IPMNs is endoscopic ultrasound (EUS). However it is more invasive and lacks specificity. On the contrary contrast-enhanced EUS has high specificity (90%) and contributes to discriminate entities (14).

Nevertheless, the most promising method in the area of diagnostics in IPMNs is endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA). This technique allows the analysis of cyst fluid using biomarkers. It is evident that only adequately trained centers can reserve this analysis, given that the specimen can be easily contaminated. EUS-FNA has the ability to distinguish mucinous from non-mucinous cysts (15, 16).

Current Practice and the Updates on the Genomic Field

Research on the field is mainly focused on the identification of biomarkers, predictive of the natural history of IPMNs, as well as diagnostic between benign and malignant pancreatic diseases. Unfortunately, no reliable and efficient biomarker has been established to serve these purposes (17).

It is commonplace among literature that the carcinoembryonic antigen (CEA) is the most commonly used biomarker for the diagnosis and follow-up of IPMN (18). CEA is a glycoprotein of the cell surface whose levels are elevated in over 60% of patients with pancreatic ductal adenocarcinoma. It is released from the peripheral cancer cell membrane and, then, is free to enter the systemic circulation. Concerning the diagnosis of IPMN, studies suggest a cut-off of 192 ng/ml. Unfortunately, CEA cannot discern benign from malignant cysts (19). Moreover, it is reported to be equally raised in mucinous cystic neoplasms and in 30% of the samples it is low (20-25). Therefore, the evaluation of serum CEA level is preferred. The sensitivity of serum CEA concerning malignant and invasive IPMNs is 18%, which is too low to be used as a screening method, especially in high-risk patients. On the other hand, the specificity of serum CEA is approximately 95%, so this marker can be used to rule-in IPMN malignancy. Serum CEA level >5 ng/ml is thought to be a potent predictor of malignancy and invasiveness. Nevertheless, it is evident that in order to decide a therapeutic plan that includes treatment modalities of increased severity, such as surgery, an elevated serum CEA level is not a definite marker.

The carbohydrate antigen 19-9 (CA19-9) is the other biomarker often used for the diagnosis of IPMNs (18, 26). It is a tumor-associated glycoprotein. Both CA19-9 and CEA are shown to be relatively adequate predictors of the diagnosis and prognosis of ductal adenocarcinoma; however, it seems that CA19-9 is better predictor of malignant status. CA19-9 levels are elevated in 85% of patients with pancreatic ductal adenocarcinoma. The sensitivity of CA19-9 is 44% and the specificity is 85%. Moreover, there is an association between the increased levels of CA19-9 and the presence of invasive IPMN. Consequently, in patients with positive tumor markers, when surgery is decided or indicated, we should not attempt limited resections (27).

Kirsten-ras (*KRAS*) is an oncogene that encodes a membrane-bound guanosine triphosphate (GTP)-binding protein. It is located on chromosome arm 12p. It is often associated with mutation at the codon 13 (1, 28). Studies do not agree on the frequency of mutations of the *KRAS* gene as it is ranged from 38.2-100% (29, 30). It is possible that this wide range is correlated to better definition of the lesions. Also, the sensitivity of the chosen screening methodology could affect the variety of these frequencies. Furthermore, as the percentage in low, intermediate and high-grade dysplasias is about 70-90%, it can be assumed that there is no significant difference in the frequency of *KRAS* mutations in variable grades of dysplasia.

The expression of *KRAS* mutation can be studied in surgical specimens, in peripheral blood and in the pancreatic

juice (28). Concerning the pancreatic juice, the expression of *KRAS* mutation is not a specific biomarker for pancreatic neoplasms as a similar expression could be found in chronic pancreatitis or other inflammatory pancreatic diseases (31). Moreover, if the mutation is present in the main tumor, the changes in the expression of *KRAS* can be found in the peritumoral region and in other IPMN lesions (31). Kobayashi *et al*. showed that between patients with and without mutation in the *KRAS* gene detected, a significant change in the diameter of the main pancreatic duct was noted, so it is possible that the frequency of mutations in the *KRAS* gene could be correlated to hyper-secretion of mucin. In addition, the pancreatobiliary subtype shows the highest frequency of *KRAS* mutations, while the intestinal subtype shows the lowest (32). It is understandable that *KRAS* could be used as a possible indicator for mucinous differentiation of pancreatic cysts. On the other hand, the lack of specific differentiation among the incidence of *KRAS* mutation in the several stages of dysplasia shows that *KRAS* cannot be used for the identification of benign and malignant IPMNs. Finally, we should report that if the CEA level is low, then the KRAS testing is extremely valuable for the presence of malignant IPMN (27, 33).

Furthermore, *GNAS* is an oncogene that encodes the guanine nucleotide-binding protein (G-protein) alpha subunit (Gs-α). It is located on chromosome arm 20q. It is often associated with mutation at the codon 201 (34). *GNAS* is a very important biomarker given the fact that *GNAS* mutations are found only in IPMNs (28, 35). One hundred percent of the intestinal type displays *GNAS* mutations, while 71% of pancreatobiliary type and 51% of gastric types shows these mutations. Moreover, the *GNAS* mutation rate in IPMN with distinct pancreatic adenocarcinoma was recently found to be significantly lower than that in IPMN without adenocarcinoma (36). *GNAS* wild-type and gastric type IPMNs were significantly associated with distinct adenocarcinomas (36). We should mention that in oncocytic IPMNs, *GNAS* mutations are not detected. In general, *GNAS* mutations are often in more advanced lesions. A recent experimental study demonstrated that activating mutations in *GNAS* and *KRAS* cooperatively promote pancreatic tumorigenesis, which recapitulates IPMN (37).

Another oncogene that has been studied is *BRAF*. *BRAF* encodes the serine-threonin kinase b-Raf. It is a compound that participates at the RAS/ MAPK pathway, which is very important for the regulation of pivotal cellular functions (38). BRAF is one of the three isoforms of Raf, that is the starting molecule of the kinase cascade (30). It is located on chromosome arm 7q. Concerning IPMNs, while the incidence of mutant *BRAF* is only 2.7%, both the changes in the Ras/Raf/MEK/ERK/MAP pathway caused by mutant *BRAF* and the *RAS* mutation are reported to significantly affect the tumor genesis of IPMNs. Furthermore, studies report that both *BRAF* and *KRAS* transformation lead to accelerated course in the progression of tumorigenesis (39).

It is widely known that telomeres are nucleoprotein structures in the ends of chromosomes responsible for the genomic stability. Telomerase, a RNA-dependent DNA polymerase, restores the reduction of telomeres as it elongates the telomeric DNA sequence, but is normally inactivated in human cells (40). The human telomerase reverse transcriptase (h*TERT*) gene is the catalytic component of telomerase. It is located on chromosome 5 (40). In general, in IPMNs, it is common that the size of telomeres is gradually decreased and h*TERT* expression is elevated in comparison to non-malignant neoplasms (41).

Another oncogene that has been evaluated in pancreatic oncogenesis is Hedgehog. Hedgehog (Hh) proteins constitute a family of secreted signaling factors that participate at the regulation of development of tissues (42). One of the genes that belong to the Hh family is sonic hedgehog (*SHH*) gene and its abnormal activity is reported in IPMNs (43). *SHH* expression is detected in 68.8% of intestinal types, 92.8% of pancreatobiliary types, 38.1% of null types and in 50% of unclassified types. Moreover, *SHH* expression can be detected in pancreatic juice from IPMN, but not in pancreatitis juice, so by detecting *SHH* expression, IPMN can be discriminated from chronic pancreatitis (44). We can assume that *SHH* activity is associated with the early stages of IPMNs and that its activation may affect the transformation from benign to malignant dysplasia in IPMN. In addition, *SHH* expression is found to correlate in metastatic progression to lymph node in malignant IPMN.

There are also investigations in the field of tumor suppressor genes. Cyclin-dependent kinase inhibitor 2A/p16 (*CDKN2A*) is a tumor suppressor gene that encodes the cyclin dependent kinase (Cdk) inhibitor p16Ink4A. It is located on chromosome 9p (45). Its inactivation due to homozygous deletion, mutation or epigenetic knock-down by methylation appears at late stages of pancreatic carcinogenesis (46). In borderline IPMNs, the inactivation of P16 protein is detected in 10-25% of the cases, while in the IPMN carcinomas this loss is described in approximately 80-100% (47). Evidently, the inactivation of P16 is the necessary but not the sufficient treaty in order to induce the alteration from non-invasive IPMN to invasive carcinoma. However, inactivation of P16 alone is reported as the most powerful biomarker in discrimination of IPMN with low-grade/intermediate dysplasia from the IPMN with carcinoma (46).

Another widely known tumor suppressor gene is *P53*. *P53* encodes the proper regulation of cell cycle. It is located on chromosome arm 17p. Studies do not agree on the accurate percentage of mutant *P53* gene in IPMN (27.3-52%); however, the consensus is that, in adenoma dysplasia, we do not detect *P53* mutation. On the other hand, *P53* mutation is found in 33.3% of borderline tumor and in 38.5% of non-

invasive carcinoma (48). The inactivation of *P53* seems to display at a late stage in the carcinogenesis of IPMN. A recent study demonstrated that NOP14 overexpression promoted cell motility, whereas NOP14 inhibition decreased invasive capacity of pancreatic adenocarcinoma cells *via P53* mutation stability that was validated as a functional target of NOP14 (49). The NOP14/mut*P53* axis suppressed *P21* expression at both the transcriptional and post-transcriptional levels *via* induction of microRNA-17-5p in pancreatic adenocarcinoma cells (49). We should mention that in IPMNs, the inactivation of *P53*, concomitant with the inactivation of the *P16*, are found in 20% of borderline tumors, 33% of the non-invasive carcinomas, in all the invasive carcinomas and in any adenoma (50). It is understandable that the transformation of IPMN to an invasive carcinoma depends crucially on the loss of action of both *P53* and *P16*.

Serine/threonine kinase 11 (*STK11*) gene encodes growth-suppressing activity and the regulation of P53-dependent apoptosis. It is located on chromosome arm 19p (51). *STK11* mutation is associated with IPMN and pancreatic ductal adenocarcinoma (52). Moreover, there is a particular correlation between *STK11* mutation and Peutz-Jegher syndrome. Loss of heterozygosity at 19p 13.3 is detected in all IPMNs from patients with this syndrome and only in 25% of patients lacking the phenotype of Peutz-Jegher syndrome (53). Knowing that patients with Peutz-Jegher syndrome have an extremely increasing risk of developing pancreatic cancer, IPMN could be a beneficial precursor of pancreatic ductal adenocarcinoma.

Another gene that has been studied is Brahma-related gene 1 (*BRG1*). *BRG1* is an ATPase that encodes a subunit of the SWI/SNF chromatin remodeling complex that makes target genes accessible to transcription factors. It is located on chromosome arm 19p (54). Concerning IPMNs, inactivation of *BRG1* is reported in 53.3% of the lesions. Interestingly, a gradual loss of *BRG1* expression is correlated to an increasing degree of dysplasia (55).

Recent studies have investigated the activity of members of the S100 family in IPMNs (56). The S100 family constitutes a group of proteins that participate in vital signaling pathways, such as the Ca^{2+} signaling network (57, 58). In IPMNs, the levels of expression of several members of this family, such as S100P, are highly elevated in bulk pancreatic tissues, than in non-neoplastic pancreatic tissues. Furthermore, in micro-dissected cells, S100P expression is reported to be increased in pancreatic ductal adenocarcinoma in relation to IPMN cells (56, 59). Interestingly, in pancreatic juice, S100P is highly expressed in IPMNs in relation to chronic pancreatitis (60). Therefore, the measurement of S100P levels in pancreatic juice could be used for the discrimination of neoplastic disease from chronic pancreatitis. Moreover, another member of this family is S100 calcium binding protein A4 (S100A4) and its expression is related to tumor metastasis (56). S100A4 is expressed only in 7.4% of adenomas and borderline dysplasias and in 42.9% of IPMN-derived carcinomas (56). We understand that S100A4 is a promising marker of malignancy, given the fact that we could use it for the diagnosis and study of IPMN. Further studies in the activity of S100 family members are needed, so that some of these compounds could be used as biomarkers of pancreatic carcinogenesis (61).

Studies have also focused on the DNA methylation in genes. It is known that cytosine phospho-guanine (CpG) islands are genomic regions with high presence of CpG sites (62). In more than 80% of the IPMNs, it is reported that tumor suppressor gene expression by promoter hypermethylation at CpG islands is silenced. Interestingly, higher grade IPMNs possess a highest number of methylated genes than lower grade IPMNs (63). Significantly, the mismatch repair genes MutL homolog 1 (h*MLH1*) and O^6-alkylguanine DNA alkyltransferase (*MGMT*) are found more frequently methylated in the higher grade IPMNs than in lower grade IPMNs (64). We should mention that, in the adenocarcinomas associated with IPMN, hypermethylation involving three or more promoter regions is found in 55% of the samples, while, in non-invasive IPMNs, 20% of the samples are found methylated in at three or more gene promoters (65). It is assumable that the pancreatic juice can be analyzed concerning the methylated DNA in order to differentiate the invasive from non-invasive IPMNs before surgery.

Other genes that have been associated with carcinogenesis are mucins. Mucins (*MUCs*) are genes that are reported to influence homeostasis and carcinogenesis. Particularly in pancreatic cancer *MUC1* and *MUC2* are showed to be specific markers of aggressive types (66). It has been shown that MUC protein expression can be measured by EUS-FNA. In IPMNs, MUC1 is detectable in 90% of tubular type invasion, while it is detectable in 8.6% of borderline IPMNs and in 35.8% of carcinoma IPMNs (67). We should mention that the detection of MUC1 in cystic fluid of IPMNs is rare (68). In general, over-expression of MUC1 is associated with invasive carcinoma. Moreover, MUC2 is a secretory mucin, associated with the regulation of cell proliferation (66). It is a very promising gene as it is not expressed in pancreatic tissue normally but it can be detected it in intestinal-type IPMN and colloid carcinomas (29, 68). Its expression is elevated gradually from adenoma/borderline IPMN to carcinoma *in situ*, to colloid carcinoma. On the other hand, in ordinary pancreatic ductal adenocarcinomas, only 1% of the samples express MUC2 (66). Concerning invasive carcinomas developed from IPMN, colloid carcinomas express MUC2 but they do not express MUC1, while only 1% of tubular adenocarcinomas express MUC2 (60% express MUC1) (66).

Recently, Das *et al*. developed a monoclonal antibody, called mAb Das-1, against a colonic epithelial phenotype.

Both the EUS-FNA cyst fluid analysis (specificity and sensitivity of 100% and 89%, respectively) and histological specimens from resected high-grade IPMNs (specificity and sensitivity of 95% and 85%, respectively) showed that Das-1 has potent reactivity (69).

Finally, we should mention microRNAs as a very promising field in the area of biomarkers. MicroRNAs (miRNAs) are small single-stranded RNA molecules that participate at the regulation of gene expression (70). Abnormal expression of miRNAs is observed both in pancreatic adenocarcinoma and its precursor lesions, such as IPMN (71). In particular, miRNA-21 (miR21) and miRNA-155 (miR155) expression is significantly up-regulated in invasive IPMNs compared to non-invasive IPMNs and normal pancreatic tissue (72). Also, in IPMNs with carcinoma *in situ*, both the expression of miR21 and miR155 is up-regulated more than in IPMN adenomas (73).

Peripheral Blood Cell

A strong link between neutrophil infiltration and malignant progression has been described, with inflammatory mediators released by these cells playing a pivotal role in the crosstalk between neoplastic and inflammatory cells (74). Recently, it was reported that tumorigenesis in the pancreas is associated with significant intra- and peri-tumoral inflammation and failure of protective immunosurveillance (75). Neutrophil activation and proliferation are indicative of TH-1 pathway of cell-mediated immunity involvement (76). Reasonably, the first phase of inflammation is correlated with increased neutrophil expression, whereas the other 2 phases should be correlated with increased lymphocyte expression (17). Thus, despite being non-specific, increased neutrophil-to-lymphocyte ratio (NLR) might be indicative of increased active inflammatory process in IPMN-derived malignancies. Peri-pancreatic lymphocyte subsets have divergent effects on tumorigenesis by either suppressing cancer growth *via* antigen-restricted tumoricidal immune responses ($CD8^+$ T-cells and Th1-polarized $CD4^+$ T-cells) or by promoting tumor progression *via* induction of immune suppression (myeloid-derived suppressor cells; MDSCs and Th2-deviated $CD4^+$ T-cells) (76, 77). Another study showed that pancreatic adenocarcinoma-infiltrating γδ-T cells are a highly influential lymphocyte subset promoting pancreatic oncogenesis and reducing survival *via* novel cross-talk with the adaptive immune compartment; Nevertheless, they may also have prognostic significance of survival and response to immunotherapeutic regimen (77). Investigators from our Institution have reported an association between tumor-associated neutrophils (TANs) and advanced IPMN lesions (78). The recent publication of Gemenetzis *et al*. (79) evaluating the correlation between NLR and platelet-to-lymphocyte ratio (PLR) values and the presence of invasive carcinoma in patients with IPMN. Despite the limitations of the study that the authors acknowledge, including its retrospective nature and the non-specificity of the results since blood is a complex fluid with high cellular turnover, the striking finding was that NLR was significantly elevated in patients with IPMN-associated invasive carcinoma in a value higher than 4 (79).

Also, preoperative NLR, in intraductal papillary mucinous carcinoma (IPMC), was significantly higher in patients with IPMC (2,510±8.4 cells/mm^3) than in patients with IPMN (2,010±7.1 cells/mm^3, p=0.0079) and healthy volunteers (1,370±3.3 cells/mm^3, p<0.0001) (80). NLR was significantly reduced after curative tumor resection. The main duct type (p=0.0231) and NLR >2.074 (p=0.0329) were independent predictors of IPMC in all patients. Combined criteria, including international consensus guidelines, CA19-9 >37 IU/ml and NLR >2.074, showed a high positive predictive value of 78 % and high specificity of 96% (80).

Table I restates the promising biomarkers in order to study the clinical management of IPMNs.

Future Perspectives

We are going through an era of individualization of surgical oncology; in fact, surgery -and anticancer research in general- is now way more personalized than ever before and different issues are treated differently. Furthermore, patients with similar conditions can be treated differently. In the case of IPMN, we believe that precision surgery would be a crucial step to improve oncological outcomes and early diagnosis since patient-specific metabolomic and genomic profile evaluation will give surgeons the opportunity to identify those individuals who will benefit from an early operation and those who will benefit from watchful waiting approach.

A recent meta-analysis determined the relationship between specific genetic alterations and malignant transformation in IPMN of the pancreas. The authors demonstrated that the expression of *hTERT* was strongly associated with malignant transformation in IPMN, consistent with up-regulation of *hTERT* as a key step in progression of IPMN to cancer (67). On the other hand, expression of *KRAS* and *MUC5AC* was common but not strongly associated with IPMN histologic progression (67). The quality criteria used here may guide future reporting of genetic markers related to malignant transformation of IPMN.

All in all, increasing evidence suggests that pancreatic cancer progression is not only caused by the intrinsic properties of tumor cells but also stimulated by host systemic and local inflammatory reactions, including immune cells and cytokines, that might develop a tolerogenic or pro-inflammatory environment that significantly facilitate tumor invasion and metastasis ability (53). Most studies on the field

Table I. *Promising biomarkers in pancreatic cystic lesions.*

Marker Name	Marker abbreviation	Function	Use in clinical practice
Carcinoembryonic antigen	CEA	Low sensitivity and elevated specificity for malignant and invasive IPMN	✔
Carbohydrate antigen 19-9	CA19-9	Differentiation of invasive from benign IPMNs	✔
Kirsten-ras	*KRAS*	Detection in approximately 50% of IPMNs	✔ (rarely)
GNAS complex locus	*GNAS*	Detection in approximately 60% of IPMNs, often in more advanced lesions	*
V-raf murine sarcoma viral oncogene homolog B1	*BRAF*	Mutation affects tumor genesis of IPMNs	
Human telomerase reverse transcriptase	h*TERT*	Highly expressed in IPMNs in comparison to non-malignant neoplasms	*
Sonic hedgehog	*SHH*	Discrimination of IPMN from chronic pancreatitis	*
Cyclin-dependent kinase inhibitor 2A/P16	*CDKN2A*	Detection of inactivation in approximately 90% of IPMNs, discrimination of low-grade IPMN from carcinoma IPMN	*
Tumor protein P53	tP53	Not detected mutation in adenoma dysplasia	
Serine/threonine kinase 11	*STK11*	Loss of heterozygosity in 100% of IPMNs in patients with Peutz-Jegher syndrome	
Brahma-related gene 1	*BRG1*	Inactivation in approximately 50% of IPMNs, correlation to dysplasia	
S100 protein	S100P	In pancreatic juice, over-expression in IPMNs in relation to chronic pancreatitis	
S100 calcium binding protein A4	S100A4	Detection in approximately 43% of IPMNs	*
MutL homolog 1	hMLH1	Highly methylated in higher grade IPMNs	
O^6 – alkylguanine DNA alkyltransferase	*MGMT*		
Mucin 1	*MUC1*	Detection in 90% of tubular type invasion and in 35.8% of IPMNs, overexpression associated with invasive adenocarcinoma	
Mucin 2	*MUC2*	Detection in intestinal-type IPMN and colloid adenocarcinomas	*
Monoclonal antibody against a colonic epithelial phenotype	mAb Das-1	Specificity for high-risk and malignant IPMNs	*
microRNAs	miR21, miR155	Up-regulation in invasive IPMNs	*
Neutrophil-to-lymphocyte ratio	NLR	Increase in patients with invasive carcinoma associated with IPMN, prediction of IPMC in patients with IPMN	*

The check mark (✔) refers to the biomarkers that are used in clinical practice. The asterisk (*) refers to the most promising biomarkers that are not utilized at the moment.

fail to elucidate the pancreatic oncogenesis pathways and mainly evaluate several biomarkers that could be indicative or epiphenomenon of many cellular or molecular processes (39). Thus, future research should focus on identifying the pathways and mechanisms of pancreatic oncogenesis and tumor progression.

Conclusion

There is an ongoing interest on the field of IPMN diagnosis and identification of these cases that are more likely to progress to malignancy. According to current guidelines, as we are still unable to clearly understand the extent of MD involvement preoperatively, we should surgically resect both the MD-IPMNs and mixed-type IPMNs. We should punctuate that, despite the increasing interest in the area of biomarkers, in clinical practice, we continue to mainly utilize two biomarkers, CEA and CA19-9. DNA analysis of pancreatic cystic fluid and genomic analysis could offer new tools to the diagnostic armamentarium in combination with EUS-FNA of pancreatic cyst fluid sampling that could be extremely useful for the discrimination of benign and malignant IPMNs. Recent data also focus on blood/serum markers that could be related to malignant potential and would be easier to collect and apply in daily practice.

All in all, recent research on IPMNs focuses on ways to distinguish low-risk from high-risk lesions and decide which patients need surgery and which could be just under surveillance. However, it is required that more studies will be realized in order to completely understand the nature of these diseases. Therefore, as IPMN might be considered as precursor of pancreatic ductal adenocarcinoma, the understanding of the molecular pathology of this neoplasm could pander other outcome in relation to the therapy of pancreatic ductal adenocarcinoma.

Conflicts of Interest

All Authors declare that they have no competing interests.

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