Functions and regulation of the PTEN gene in colorectal cancer

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INTRODUCTION

Phosphatase and TENSin homolog deleted on chromosome 10 (PTEN), known also as mutated in multiple advanced cancer 1 (MMAC1), is a tumor suppressor gene located at chromosome 10q23.11 and encodes for a 403-amino acid protein that possesses both lipid and protein phosphatase activities. The crystal structure of PTEN revealed two major functional domains (a phosphatase domain and a C2 domain) and three structural regions [a short N-terminal phosphatidylinositol (PI)-4,5-bisphosphate (PIP2) binding domain and a C-terminal tail containing PEST sequences and a PDZ-interaction motif] (Figure 1) (1). The PTEN protein is principally involved in the homeostatic maintenance of PI3K/Akt signaling originating from EGFR activation (or activation of other tyrosine kinase receptors or G-protein-coupled receptors) (Figure 2). Its typical function consists of the dephosphorylation of the lipid-signaling second messenger PI 3,4,5-triphosphate (PIP3), a lipid product of the PI-3-kinase (PI3K) (2), thereby directly antagonizing the PI3K function and blocking therefore the activation of downstream signaling events, including PDK1 (akt) and akt/mammalian target of rapamycin (mTOR). The opposite biochemical reaction is catalyzed by PI3Ks, which are associated with cell growth and cell survival (Figure 2). Thus PTEN, which counteracts PI3Ks activity, is involved in inhibition of cell cycle progression, induction of cell death, modulation of arrest signal, and stimulation of angiogenesis (3).

The lipid phosphatase activity of PTEN is the best-characterized physiological function contributing to the tumor suppressor function of PTEN. As no other redundant and/or compensatory family members have been found, PTEN is the only known lipid phosphatase counteracting the PI3K pathway. It is not surprising that loss of PTEN function, resulting therefore in increased PIP3 and persistent activation of PI3K effectors, has an important impact on multiple aspects of cancer development such as cell proliferation, apoptosis resistance, angiogenesis, metabolism regulation, genomic instability, stem cell self-renewal, cellular senescence, and cell migration and metastasis (4, 5).

Phosphatase and TENSin homolog deleted on chromosome 10 (PTEN) is a tumor suppressor gene located at chromosome 10q23.31, encoding for a 403-amino acid protein that possesses both lipid and protein phosphatase activities. The main function of PTEN is to block the PI3K pathway by dephosphorylating phosphatidylinositol (PI) 3,4,5-triphosphate to PI-4,5-bisphosphate thus counteracting PI3K function. PTEN inactivation is a frequent event in many cancer types and can occur through various genetic alterations including point mutations, large chromosomal deletions, and epigenetic mechanisms. In colorectal cancer (CRC) PTEN is altered through mixed genetic/epigenetic mechanisms (typically: mutations and promoter hypermethylation or 10q23 LOH and promoter hypermethylation), which lead to the biallelic inactivation of the protein in 20–30% of cases. The role of PTEN as a prognostic and predictive factor in CRC has been addressed by relatively few works. This review is focused on the report and on the discussion of the studies investigating these aspects. Overall, at the moment, there are conflicting results and, therefore it has not been clarified whether PTEN might play a prognostic role in CRC. The same is valid also for the predictive role, leading to the fact that PTEN evaluation cannot be used in routinely diagnosis for the early identification of patients who might be addressed to the treatment with EGFR-targeted therapies, at odds with other genetic alterations belonging to EGFR-downstream pathways. The reason of discordant results may be attributable to several issues: (1) the size of the analyzed cohort, (2) patients inclusion criteria, (3) the methods of assessing PTEN alteration. In particular, there are no standardized methods to evaluate this marker, especially for immunohistochemistry, a technique suffering of intra and inter-observer variability due to the semi-quantitative character of such an analysis. In conclusion, much work, especially in large and homogeneous cohorts of cases from different laboratories, has to be done before the establishment of PTEN as prognostic or predictive marker in CRC.

Keywords: PTEN, colorectal cancer, mutation, immunohistochemistry, prognosis, predictive, EGFR-targeted therapies
In its inactive state, PTEN is phosphorylated on a cluster of serine and threonine residues located on its C-terminal tail, leading to a closed PTEN state and maintaining PTEN protein in a stable conformation. When PTEN is being activated, dephosphorylation of its C-terminal tail opens its phosphatase domain, thereby increasing PTEN activity. Meanwhile, the open state of PTEN is more susceptible to ubiquitin-mediated proteasomal degradation (4, 6); therefore, this mechanism is a negative feedback leading to decreasing and switching off the effect of PTEN, in absence of extracellular stimuli (e.g., presence of insulin, growth factors, chemokines). PTEN is also recruited to the membrane by interacting with a number of membrane-anchored proteins, via its C-terminal PDZ-interaction motif (7). In addition, PTEN mono- ubiquitination controls PTEN nuclear entry. In some tumors, the subcellular localization of PTEN protein seems to mediate its activity (8). The absence of PTEN has been reported to be associated with more aggressive diseases and with high degree of neoplastic transformation, suggesting an important nuclear function for PTEN in tumor suppression (9, 10).

A number of factors have been shown to transcriptionally regulate PTEN mRNA [reviewed by Song et al. (3)], including peroxisome proliferation-activated receptor γ (PPARγ), early growth-response protein 1 (ERG1), and p53. PTEN mRNA is also post-transcriptionally regulated by PTEN-targeting microRNAs such as miR19 and miR21 and is now emerging that also PTEN pseudogene (PTENP1) may be able to regulate PTEN expression (5).

PTEN loss of function occurs in a wide spectrum of human cancers through various genetic alterations including point mutations (missense and nonsense mutations), large chromosomal deletions ( homozygous/heterozygous deletion, frameshift, inframe deletion, and truncation), and epigenetic mechanisms as hypermethylation of the PTEN promoter region. In addition, PTEN could be inactivated by other non-structural alterations affecting transcript stability, protein stability, and differential subcellular compartmentalization (4, 5, 8).

Despite its serine, threonine and tyrosine phosphatase activity, the lipid phosphatase function of PTEN has been shown to be the major driving force in tumor suppression. In fact the G129E mutation, observed in cancer specimens and abrogating the lipid phosphatase activity but maintaining its protein phosphatase activity, leads to PTEN tumor suppressor function inactivation in vitro (11–13).

Loss of heterozygosity at 10q23 occurs frequently in many sporadic tumors at advanced stage; for example, approximately 70% glioblastoma and 60% advanced prostate cancer are characterized by loss of that region. Somatic mutation in the second allele of PTEN, which results in biallelic inactivation, occurs in 25–40% of glioblastomas.

Somatic mutations of PTEN have been identified as the main mechanism of inactivation in many tumor types, particularly those of the endometrium, brain, skin, and prostate. The tumor suppressor function of PTEN is usually abrogated following mutations occurring in its phosphatase domain (encoded by exon 5): typically, the C124S mutation (that abrogates both lipidic and protein phosphatase activity) and the G129E mutation (that abrogates only lipid phosphatase activity) (4, 14). Although the N-terminal phosphatase domain is principally responsible for PTEN physiologic activity, approximately 40% of PTEN tumorigenic mutations may occur in the C-terminal C2 domain (corresponding to exons 6, 7, and 8) and in the tail sequence (corresponding to exon 9), encoding for tyrosine kinase phosphorylation sites important for maintaining PTEN function and protein stability (3, 4, 8, 15). In endometrial carcinoma, glioblastoma, and lymphoma, cancer-specific mutations have been found also in the PIP2-binding region, thus highlighting the importance of this motif for the functionality of PTEN protein (16, 17). In addition to missense mutations, a number of nonsense and frameshift mutations have been described leading to truncated PTEN proteins lacking the C-terminal tail and the PDZ-interaction motif, important domains for PTEN protein stability and recruitment to the membrane, without which PTEN is biochemically inactive (5, 8).
However, in sporadic tumors, loss of heterozygosity of *PTEN* occurs at a much higher frequency than biallelic inactivation. It remains unclear whether haploinsufficiency of *PTEN* provides a selective growth advantage in tumors lacking a second hit in the remaining *PTEN* allele. Evidence for a role of *PTEN* haploinsufficiency was demonstrated in a mouse model of prostate cancer in which the dosage of *PTEN* was inversely correlated to the severity of tumor phenotype (18).

Finally, *PTEN* can be altered also in inherited syndromes. That is the case of the Cowden disease whose patients tend to develop breast, thyroid, and skin tumors. In these types of tumors *PTEN* exerts its role in the initiation and in the progression of cancer (3, 12, 19).

**PTEN IN COLORECTAL CANCER**

In CRC *PTEN* is altered through a mixed genetic/epigenetic mechanism (typically: mutations and promoter hypermethylation or 10q23 LOH and promoter hypermethylation), which leads to the biallelic inactivation of the protein in 20–30% of cases.

*PTEN* expression and mutational rate was reported to be lower in left-sided (distal) CRC in comparison to right-sided (proximal) cancers (20–22). This finding may be related to different genetic mechanisms underlying the tumorigenesis of proximal and distal sporadic CRCs. Cancers arising in right colon are usually characterized by microsatellite instability (MSI), whereas those arising in the distal colon and in the rectum are very often characterized by chromosomal instability (CIN). Therefore, it can be argued that *PTEN* alterations may be linked to MSI and to the mechanisms leading to MSI (including high frequency of promoter hypermethylation, the main mechanism of mismatch repair genes silencing, whose absence of function is directly responsible of MSI). Consistent with this hypothesis, Day and colleagues found that *PTEN* mutations, identified in about 6% out of 744 stage I-IV CRC, were associated with mucinous histology, MSI, CpG island methylator phenotype, and *BRAF* mutations (22). Furthermore, other reports demonstrated a direct association between *PTEN* mutations and MSI, suggesting that the *PTEN* gene is a target of genomic instability in MSI colorectal tumorigenesis (23–25). In particular, Zhou and colleagues found that among 11 HNPPC CRC, 32 MSI sporadic cancer, and 39 microsatellite stable tumors, *PTEN* somatic mutations were found in 18, 13, and 0% of cases respectively, and *PTEN* loss of expression (evaluated by IHC) in 31, 41, and 17%, respectively. The majority of somatic mutations occur in the two 6(A) coding mononucleotide tracts, suggesting an etiological role of the deficient mismatch repair system (25). Moreover, it was also reported that *PTEN* promoter hypermethylation is a frequent event in sporadic CRC with MSI and may represent an important epigenetic mechanism of *PTEN* inactivation in this setting (26).

Overall, although another study did not confirm this association (because gene mutations and LOH were found in about 20 and 17% of sporadic CRC respectively, all but one of which were microsatellite stable) (27), we can assume that *PTEN* alterations and MSI are correlated.

In addition to *PTEN* level, the PI3K pathway can be altered following mutations in genes encoding for PI3K proteins, typically in *PIK3CA* gene. Therefore, it has been proposed that *PTEN* alterations and *PIK3CA* mutations may be mutually exclusive. However, this concept has not been deeply demonstrated as few studies investigating this topic showed conflicting results. There is in fact a clear evidence that mutations in multiple components of the PI3K pathway are not necessarily redundant. Although activating mutations in *PI3K* and loss of *PTEN* function both enhance PI3K signaling, these alterations seem not to cover equivalent functions. For example, in endometrial cancer, mutations in *PTEN* and *PIK3CA* both occur frequently and often concomitantly within the same tumor, indicating a potential additive or synergistic effect (28–30).

As for the other genetic alterations mainly occurring in CRC, it has been demonstrated that loss of *PTEN* expression measured by IHC co-occurs with *KRAS* and *BRAF* mutations and with EGFR polysomy (31), whereas *PTEN* and *TP53* mutations seem to be mutually exclusive (27).

**PROGNOSTIC ROLE OF PTEN**

The role of *PTEN* as a prognostic factor in CRC has been addressed by relatively few works.

Although accumulating evidence has strongly suggested that *PTEN* is a crucial factor in various central processes of cancer development, and although in several tumor types (e.g., non-small-cell lung cancer, prostate and breast cancer) *PTEN* protein status has been correlated with poor prognosis, the association between *PTEN* expression and clinical parameters in CRC is still controversial. The studies reporting the clinical impact of *PTEN* alterations on patient outcome in CRC are here summarized. Several of these studies suggest an association between loss of *PTEN* protein expression with advanced disease, liver metastasis, and poor patient survival, whereas other works do not find such an association (Tables 1 and 2).

One of the first paper reporting an association between *PTEN* alteration and tumor aggressiveness was published in 2001 and examined *PTEN* somatic mutations in a series of 36 sporadic CRC. The authors found that *PTEN* gene mutations were detected only in patients with locally advanced or metastatic CRC (32).

The majority of the next studies have been performed by analyzing *PTEN* protein expression by IHC assay, the most effective way to assess the loss of *PTEN* function by any mechanism (LOH, somatic mutation, or promoter epigenetic silencing). In fact, it has been reported that all tumors with *PTEN* gene alterations (mutation and/or deletion) showed a reduction or absence of *PTEN* expression evaluated by IHC, and this finding was correlated with advanced stage of disease (33). This association was confirmed by Sawai and colleagues, who demonstrated that *PTEN* loss was significantly correlated with local recurrence, advanced TNM stage (*p* < 0.01), lymph node metastasis (*p* < 0.05) and with lower 5-year survival rate (*p* = 0.012), indicating a link between *PTEN* deregulation and CRC aggressive phenotype (34). A positive association of *PTEN* expression with histological grade and distant metastasis was also demonstrated by Lin and colleagues (35). Similarly, Li and co-workers, by examining nuclear *PTEN* protein expression on tissue microarray in 327 CRC, found that low level of *PTEN* protein expression was positively correlated with tumor size, depth of invasion, lymphatic invasion, lymph node metastasis, and higher tumor staging (*p* < 0.05).
Table 1 | List of papers finding a positive correlation between PTEN loss and prognosis.

| Author                  | No. | Type of tissue                                           | Method                  | % PTEN alteration |
|-------------------------|-----|---------------------------------------------------------|-------------------------|-------------------|
| Dicuonzo et al. (32)    | 36  | Frozen CRC                                             | Sequencing              | 17% mutations     |
| Nassif et al. (33)      | 41  | Frozen normal tissue and CRC                           | Sequencing, LOH, IHC    | 19% mutations     |
|                         |     |                                                        |                         | 17% LOH           |
|                         |     |                                                        |                         | 70% reduction or loss of expression (IHC) (cytoplasm and nuclear staining) |
| Sawai et al. (34)       | 69  | with liver metastasis; 70 without liver metastasis     | IHC                     | 75.4% weak expression (cytoplasm and nuclear staining)  |
| Lin et al. (35)         | 139 | FFPE TMA CRC                                           | IHC                     | 7% weak or loss expression (cytoplasm staining)  |
| Li et al. (36)          | 327 | FFPE TMA CRC                                           | Sequencing, IHC         | 29% weak or loss of expression (PTEN immunoreactivity localized in the nucleus)  |
| Jang et al. (37)        | 482 | FFPE TMA CRC                                           | IHC                     | 50% loss of expression  |
| Jin et al. (38)         | 68  | FFPE CRC                                               | IHC                     | 67.6% loss of expression (cytoplasm and nuclear staining)  |
| Atreya et al. (39)      | 56  | FFPE mCRC                                              | IHC                     | 12.3% loss of expression (cytoplasm and nuclear staining)  |
| Bohn et al. (40)        | 307 | FFPE TMA CRC                                           | FISH                    | 8.8% gene loss    |

No.: number of patients; CRC: colorectal cancer; FFPE: formalin-fixed paraffin embedded; FISH: fluorescent in situ hybridization; IHC: immunohistochemistry; LOH: loss of heterozygosity; mCRC: metastatic colorectal cancer; TMA: tissue microarray.

Table 2 | List of papers finding no correlation between PTEN loss and prognosis.

| Author                  | No. | Type of tissue        | Method                  | % PTEN alteration |
|-------------------------|-----|-----------------------|-------------------------|-------------------|
| Colakoglu et al. (21)   | 76  | FFPE CRC              | IHC                     | 5% loss of expression; 67% weakly moderate positive expression (cytoplasm staining) |
| Eklöf et al. (41)       | 197 and 414*| FFPE CRC            | IHC                     | 12.5 and 14% loss of expression (cytoplasm staining) |
| Price et al. (42)       | 302 | FFPE advanced CRC     | Taqman copy number assay | 38.7% loss     |
| Day et al. (22)         | 1093| FFPE stage-Iv CRC    | Sequencing              | 5.8% mutations    |

*Separate cohort; No.: number of patients; CRC: colorectal cancer; FFPE: formalin-fixed paraffin embedded; IHC: immunohistochemistry.

In addition, univariate and multivariate analysis indicated that patients characterized by PTEN loss of protein expression had a shorter survival than patients with a normal expression of PTEN (36).

Another study performed on 482 CRC revealed that PTEN protein expression (evaluated again on a tissue microarray) was associated with poor overall survival (OS) and disease-free survival \((p = 0.03\) and \(p = 0.046\), respectively), although in multivariate analysis, a significant difference was observed only in patients with stage II of disease (37).

Jin and colleagues by evaluating the prognostic value of PTEN, STAT3, and VEGF-C protein expression by IHC in 68 cases of CRC, showed that PTEN expression was correlated with pathological grade, but not with tumor size, lymph node metastasis, or clinical stage. Moreover the 3- and 5-years survival rates of patients normally expressing PTEN were significantly higher than those of patients with a PTEN-negative tumor (38).

In a very recent study conducted on 56 patients affected by a metastatic disease, PTEN protein expression was analyzed by an optimized PTEN IHC assay recently developed and it was found that the median OS of patients whose tumors did not express PTEN was 9 months, compared to 49 months for patients with a normal expression of PTEN \([HR = 6.25, 95\% \text{ CI}, p = 0.0023]\). The association of absence of PTEN expression with increased risk of death remained significant in multivariate analysis \((\text{HR} = 6.31, 95\% \text{ CI}, p = 0.0023)\) (39).

Finally, the positive correlation between worse prognosis and PTEN alteration was also found after the analysis of genetic lesions. Through the evaluation of PTEN deletion and gene rearrangements by FISH on 307 CRC, the authors confirmed an association...
between PTEN alteration with reduced patient survival in univariate and multivariate analyses in rectal cancer (p = 0.012, HR 2.675; 95% CI) but not in colon cancer (40).

On the contrary with respect to the results obtained by the studies reported above, Colakoglu and colleagues, by investigating 76 CRC patients, found no correlation between PTEN immunohistochemical status and patient survival, tumor grade, TNM stage, lymphatic invasion, and liver metastasis (21), although they found a significant association between PTEN loss and local recurrence. Another study investigating the prognostic role of KRAS, BRAF, PIK3CA mutations, and PTEN expression in two separate CRC cohorts of 197 and 414 patients respectively, observed absence of correlation between PTEN status and prognosis by analyzing each molecular marker separately (41). The prognostic value of PTEN was also explored through the evaluation of PTEN gene copy number alteration (CNA) assessed by a Taqman assay by Price and colleagues in a cohort of 302 patients with advanced CRC enrolled in the AGITG MAX trial, a randomized Phase III trial of capecitabine ± advanced CRC enrolled in the AGITG MAX trial, a randomized Phase III trial of capecitabine ± bevacizumab or mitomycin C. The authors did not find any correlation between PTEN status and progression free survival (PFS) or OS in multivariate analysis (42). The absence of association with prognosis in stage II and III CRC was also supported by the work of Day and colleagues who analyzed PTEN mutations in a large cohort of sporadic CRC (22).

In conclusion, at the moment there are no clinical data clearly supporting the notion of PTEN alteration as a prognostic factor in CRC.

### Table 3 | List of papers investigating the predictive role of PTEN in CRC treated with EGFR-targeted therapies cetuximab or panitumumab

| Author               | No. | Type of tissue                  | Method         | % PTEN alteration and clinical response                                                                 |
|----------------------|-----|---------------------------------|----------------|---------------------------------------------------------------------------------------------------------|
| Frattini et al. (44) | 27  | FFPE mCRC                       | IHC            | 100% PTEN-negative patients were NR (p < 0.001)                                                         |
| Sartore-Bianchi et al. (46) | 81  | FFPE mCRC                       | IHC            | 97% PTEN-negative patients were NR (p = 0.001)                                                           |
| Perrone et al. (47)  | 32  | FFPE mCRC                       | Sequencing, FISH | All patients with a decreased PTEN gene copy number or with PTEN mutation were NR                       |
| Razis et al. (48)    | 72  | FFPE mCRC                       | IHC and FISH   | PTEN gene deletion detected only by FISH associated with no response                                    |
| Loupakis et al. (49) | 102 | FFPE mCRC (primary and metastatic lesion) | IHC            | 95% PTEN-negative patients were NR. Association with clinical response found only in the metastatic lesion |
| Negri et al. (50)    | 50  | FFPE mCRC (primary and metastatic lesion) | Immunofluorescence | 100% PTEN-negative patients were NR (p < 0.05). Association with clinical response found only in the metastatic lesion |
| Tol et al. (51)      | 559 | FFPE mCRC                       | IHC            | Loss of PTEN expression observed in 42% but not associated with response                               |
| Ulivi et al. (52)    | 67  | FFPE mCRC                       | IHC            | Loss of PTEN expression observed in 60% but not associated with response                               |
| Laurent-Puig et al. (53) | 162 | FFPE mCRC                       | IHC            | Loss of PTEN expression observed in 19% but not associated with response                               |

No: number of patients; CRC: colorectal cancer; FFPE: formalin-fixed paraffin embedded; FISH: fluorescent in situ hybridization; IHC: immunohistochemistry; mCRC: metastatic colorectal cancer; NR: non-responder to anti-EGFR therapies.
According to the reported results, the role played by PTEN as a prognostic marker in CRC is still a matter of debate. Discordant results have been reported and this fact could be attributed to several issues: (1) the size of the analyzed cohort, (2) patients inclusion criteria, (3) the methods of assessing PTEN alteration. For the latter point, it should be noted that the major-
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