

Prognostic role of neuroendocrine differentiation in prostate cancer, putting together the pieces of the puzzle

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Abstract: Neuroendocrine (NE) differentiation is a common feature in prostate cancer (PC). The clinical significance of this phenomenon is controversial; however preclinical and clinical data are in favor of an association with poor prognosis and early onset of a castrate resistant status. NE PC cells do not proliferate, but they can stimulate the proliferation of the exocrine component through the production of paracrine growth factors. The same paracrine signals may favor the outgrowth of castrate adapted tumors through androgen receptor dependent or independent mechanisms. Noteworthy, NE differentiation in PC is not a stable phenotype, being stimulated by several agents including androgen deprivation therapy, radiation therapy, and chemotherapy. The proportion of NE positive PC, therefore, is destined to increase during the natural history of the disease. This may complicate the assessment of the prognostic significance of this phenomenon. The majority of clinical studies have shown a significant correlation between NE differentiation and disease prognosis, confirming the preclinical rationale. In conclusion the NE phenotype is a prognostic parameter in PC. Whether this phenomenon is a pure prognostic factor or whether it can influence the prognosis by favoring the onset of a castrate resistance status is a matter of future research.

Keywords: prostate cancer, prognosis, neuroendocrine differentiation

Introduction

Neuroendocrine (NE) differentiation, that is usually determined by immunoreactivity for NE markers [eg, chromogranin A (CgA), neuron specific enolase (NSE)], is a common feature of prostatic adenocarcinomas.¹⁻⁴ Pure NE prostate cancers (PCs), such as small cell cancers, are rare and aggressive tumors; primary carcinoids are less aggressive and exceptional. In most cancers, the NE component coexists in the context of the non-NE component.¹ Theoretically, the spectrum of tumors having mixed divergent differentiation along NE and non-NE lineages could display a variable extension of the two components, potentially ranging from 1 to 99%.⁵ In the majority of PCs the NE component occupies a small portion of the tumor that does not exceed 5% of the overall tumor mass.¹ Only occasionally does it exceed the quota of 30%.⁵ The clinical significance of the coexistence of the NE phenotype in the context of classic prostate adenocarcinoma is still controversial, but several examples of preclinical and clinical data have shown that this phenomenon is correlated with a poor prognosis and a greater chance of developing a castrate resistant status.² In this paper, we review the biological rationale and the data supporting and not supporting the negative prognostic role of NE differentiation in prostate adenocarcinoma.

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Origin of NE differentiation in PC

Normal prostate glands harbor NE cells with the dual properties of endocrine cells and neurons that are widely distributed in normal prostatic acini and ducts. It is thought that these cells may have a regulatory role of the exocrine cells via a variety of secretory products.²

The origin of NE PC cells is not fully understood, but accumulated evidence has revealed that the biological characteristics of these cells are quite different from the normal NE prostate cells.⁶ Several dissimilarities have been observed: 1) immunohistochemical studies have shown that NE cells in the normal prostate gland express basal cell markers, while NE PC cells exhibit characteristics of luminal secretory cells;^{2,7,8} 2) NE cells from a normal prostate do not express anti-apoptotic Bcl-2 protein, while studies in archival PCa samples show a correlative relationship between the expression levels of Bcl-2 and neuron-specific enolase;⁹ 3) α -methylacyl-CoA racemase (AMACR), an enzyme involved in the β -oxidation of fatty acids, is expressed in NE PC cells, but not in non-NE prostate tumor cells nor in normal prostatic cells including NE cells.⁷ For this reason, the term 'NE-like' PC cell has been suggested to define this population of cancer cells.⁶

There are a number of theories that suggest a point of origin for these cells.¹⁰ One theory postulates that NE differentiation originates from neural crest cells,^{11,12} which give rise to cells containing neurosecretory granules known as 'amine precursor uptake decarboxylase'; another theory postulates that these cells could be the final dedifferentiation product of typical adenocarcinoma, based on a model of 'divergent differentiation'.¹³ This model suggests that NE cells might be derived from adenocarcinoma cells by a process of transdifferentiation, in response to microenvironmental changes in the hormonal and growth factor milieu. The finding that NE and exocrine tumor cells from radical prostatectomies share identical genetic profiles is consistent with this hypothesis.¹⁴

Recently, Palapattu et al have observed that in human PC cell lines, the expression of CD44, a marker of lymphocytes and cancer stem cells, is associated with cells of NE phenotype.¹⁵ In addition, NE tumor cells in human PC tissues were found to be virtually all positive for the CD44 antigen, and CD44 positive PC cells were all NE tumor cells. These results would suggest that NE cells may themselves represent the stem/progenitor cells for the bulk differentiated, secretory-type cancer cells. This interesting third hypothesis deserves confirmation.

Moreover, the NE differentiation in PC is not a stable phenotype. Existing evidence supports a model in which NE-like cells arise from prostate tumors following tumor cell exposure to one or more differentiation factors.^{6,16} Several *in vitro* experiments have demonstrated a transdifferentiation of PC cell lines into the NE phenotype in response to interleukin-6,^{17,18} AMP-inducing agents such as epinephrine,^{19,20} androgen deprivation,²¹ and genistein.²² With respect to the role of soluble cytokines in inducing NE differentiation, the mechanism may be more complex, involving the pleiotrophic activity of TGF- β signaling pathway.²³ The stimulation of prostate NE differentiation by androgen deprivation has been confirmed *in vivo*, either in the animal model²⁴ or in humans. In patients with early PC, an increase of NE cells was shown in matched tumor samples collected before and after androgen deprivation therapy (ADT) administered in a neoadjuvant setting.²⁵ While in patients with advanced disease, increased CgA serum levels were repeatedly observed after androgen deprivation therapy.^{26,27}

The stimulatory effect of the NE phenotype by androgen deprivation accounts at least in part in the NE cell enrichment observed in the castrate resistant disease.^{28,29}

Moreover, it has been repeatedly observed that prostate adenocarcinomas with NE differentiation can change phenotype during androgen deprivation, acquiring characteristics of small cell carcinoma and therefore becoming castrate resistant.^{30,31} These data are consistent with an unstable phenotype of PC with NE differentiation under androgen deprivation.

Androgen deprivation therapy, however, is probably not the only antineoplastic therapy that can modulate NE differentiation. An *in vitro* study has recently observed that fractionated ionizing radiation (IR) induces differentiation of lymph node carcinoma of prostate (LNCaP) PC cells into NE-like cells.³² In a further *in vivo* study performed in the transgenic adenocarcinoma of mouse prostate (TRAMP) model, chemotherapy with docetaxel was able to induce NE differentiation, and the incidence of this phenomenon was similar to that obtained with androgen deprivation.³³ Interestingly, the combination of both therapies, that is commonly employed in castrate resistant patients, was synergistic in inducing the NE phenotype.³³ Noteworthy, NE transdifferentiation *in vitro* is a reversible phenomenon, meaning that NE PC cells can potentially lose the NE phenotype upon withdrawal of inducing agents.³⁴

The signaling pathways involved in the NE differentiation of PC are not fully understood. Wu and Huang demonstrated

that the PI3K-AKT-mTOR pathway is critically involved in this process.³⁵

An essential role of the Notch signaling pathway, and specifically of hASH1 (human achaete-scute homolog-1 transcription factor), has been reported in determining the NE phenotype of normal and neoplastic tissues.³⁶⁻⁴² Our group has recently observed that hASH1 is also a critical factor involved in NE differentiation in PC, with special reference to patients treated with androgen-deprivation therapy.⁴³

Mori et al evaluated the effect of various NE differentiation-inducing factors on the gene signature of LNCaP cells *in vitro*, using GeneChip® (Affymetrix, Santa Clara, CA) arrays.⁴⁴ They observed that, despite some overlap, each NE transdifferentiation inducing treatment was associated with a changed expression of a unique set of genes. This observation adds complexity in the molecular mechanisms involved in the NE transdifferentiation process seen in PC.

Mechanisms by which NE differentiation can influence the prognosis of prostate adenocarcinoma

Prostate adenocarcinoma with marked NE differentiation tends to be poorly differentiated and more aggressive. Several studies have shown that NE differentiation in PC samples and serum chromogranin A levels directly correlate with a greater stage and Gleason score, but not with serum prostate-specific antigen (PSA).⁴⁵⁻⁴⁸ In a study exploring the prognostic role of gene profiling expression, chromogranin A was found to be one of the five genes that were reported to serve as outcome predictors for tumor recurrence.⁴⁹

In a paper by Tarle et al the acquirement of NE differentiation during maximal androgen blockade in PC patients was associated with an increased incidence of bone metastases.⁵⁰

Noteworthy, neoplastic NE cells do not show evidence of cell proliferation. Bonkhoff et al have repeatedly demonstrated that NE PC cells lack the proliferation-associated Ki67 antigen.^{51,52} These data suggest that the aggressiveness of PC with marked NE features cannot be explained by cell kinetic properties of the endocrine component.

NE PC cells produce a variety of neuropeptides and biogenic amines such as cholecystokinin (CCK), bombesin, gastrin-releasing peptide, histamine, neuropeptide Y, parathyroid hormone-related protein, proadrenomedullin N-terminal peptide, serotonin, thyroid stimulating hormone-like peptide; most of them are notorious growth

factors for neoplastic cells.² An interesting hypothesis, depicted in Figure 1, is that NE PC cells can stimulate the non-NE component via paracrine and endocrine signals, thus making the exocrine component more aggressive. Consistent with this hypothesis is the greater Ki67 expression observed in exocrine PC cells close to NE cells, as opposed to their counterpart.^{51,52} In addition, it has been observed that NE PC cells can produce vascular endothelial growth factor (VEGF)⁵³⁻⁵⁵ and the anti-apoptotic substance, survivin.⁵⁶ A higher density of microvessels, in fact, has been observed around nests of NE cells compared to areas of PC with no signs of NE differentiation.^{53,54} Also hypoxia-inducible factor-1 α (HIF-1 α), one of the major transcription factors which has been shown to upregulate the expression of VEGF in response to tissue hypoxia, was recently identified in a subset of benign and malignant prostate NE cells.⁵⁷

The overexpression of Bcl-2 proto-oncogene involved in apoptosis is highly correlated with cancer progression and androgen independence. It has been revealed that malignant cells that express Bcl-2 are localized in close proximity to NE tumor cells.⁵⁸ All these data suggest that the poor prognostic significance associated to the NE differentiation implies a complex interplay between the NE and exocrine component of PC.

NE differentiation and resistance to androgen deprivation

Cumulative evidence suggests that NE differentiation of CaP may be a cofactor involved in tumor progression and androgen independence.

Characteristics of the NE PC cells intermixed with a predominance of non-NE cells are the lack of detectable androgen receptor (AR) and PSA gene expression, genes that are otherwise highly expressed in prostate epithelial cells with secretory functions regulated by androgens.^{59,60} NE PC cells therefore cannot be modulated by androgen deprivation therapy.

Various mechanisms have been postulated to account for the conversion of exocrine adenocarcinoma of the human prostate (CaP) into a castration-resistant state, the emerging theme is that the tumor is still dependent on AR signaling. The proposed mechanisms that explain continued AR signaling include AR gene amplification, resulting in a response to low levels of circulating androgens, the local synthesis/concentration of androgens, AR mutations that allow activation by antiandrogens or weak androgens, AR activation by growth factors/kinase pathways, and/or changes in AR coregulators.⁶¹

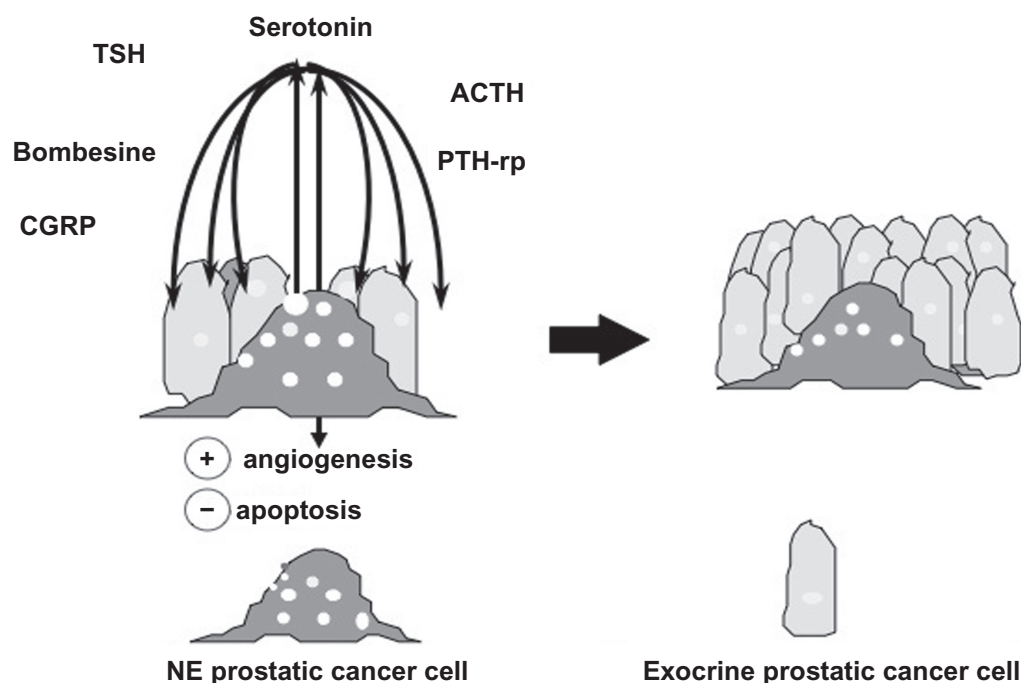


Figure 1 Neuroendocrine prostate cancer cells can stimulate the proliferation of non-neuroendocrine prostate cancer cells via paracrine and endocrine signals.

Abbreviations: TSH, thyroid stimulating hormone; CGRP, calcitonin gene-related peptide; ACTH, adrenocorticotrophin; PTHrP, parathyroid hormone-related protein; NE, neuroendocrine.

The question is how NE differentiation can interfere with these mechanisms.

It has been shown that NE secretory proteins can activate the NF- κ B pathway in PC cells, and activation of NF- κ B signaling is sufficient to maintain androgen-independent growth of PC via regulation of AR action.⁶²

A paper recently published has shown that neuropeptides can transmit their signals through G protein-coupled receptors, which are often overexpressed in PC, and aberrantly activate the AR in the absence of androgens.⁶³ The authors developed an autocrine neuropeptide model by overexpressing gastrin related peptide (GRP) (one of the most studied neuropeptides in prostate carcinoma) in LNCaP cells, and the resultant cell line, LNCaP-GRP, exhibited androgen-independent growth with enhanced motility *in vitro*. When orthotopically implanted in castrated nude mice, LNCaP-GRP produced aggressive tumors, which expressed GRP, prostate-specific antigen, and nuclear-localized AR. Chromatin immunoprecipitation studies of LNCaP-GRP clones suggested that GRP activates and recruits AR to the cognate promoter in the absence of androgen.

On the basis of these data we can postulate that NE PC cells may contribute to the outgrowth of 'castration-adapted' tumors via the production of paracrine signals that interact with the exocrine PC cells by AR dependent or independent mechanisms.

One interesting paper provides a direct demonstration of this theory.¹⁶ In this study, LNCaP cells were engineered to induce acquisition of NE-like characteristics and loss of mitotic activity. Clonal NE cells enhanced the growth of prostate tumor cells in anchorage dependent and anchorage-independent *in vitro* assays as well as the growth of prostate tumor xenografts *in vivo*, with the greatest effects seen under conditions of androgen deprivation.¹⁶ This elegant study confirms that NE-like cells of prostatic tumors have the potential to enhance androgen independent tumor growth in a paracrine manner, thereby contributing to progression of the disease particularly in the androgen deprivation condition.

As previously mentioned, NE differentiation in PC is destined to increase during androgen deprivation therapy, and this phenomenon could enhance the onset of a castrate resistant phase as depicted in Figure 2.

Finasteride is a 5- α reductase inhibitor that leads to an intraprostatic dihydrotestosterone depletion. A recent explorative paper has shown that finasteride administered to patients with prostate hypertrophy was able to favor elevation of circulating CgA serum levels.⁶⁴ In the PC prevention trial (PCPT), finasteride administered to healthy men was able to reduce the risk of PC by 25% with respect to placebo, but with an apparent increased risk of high-grade disease.⁶⁵ Even though an update of this study failed to confirm a significant difference in the proportion of high grade disease between the

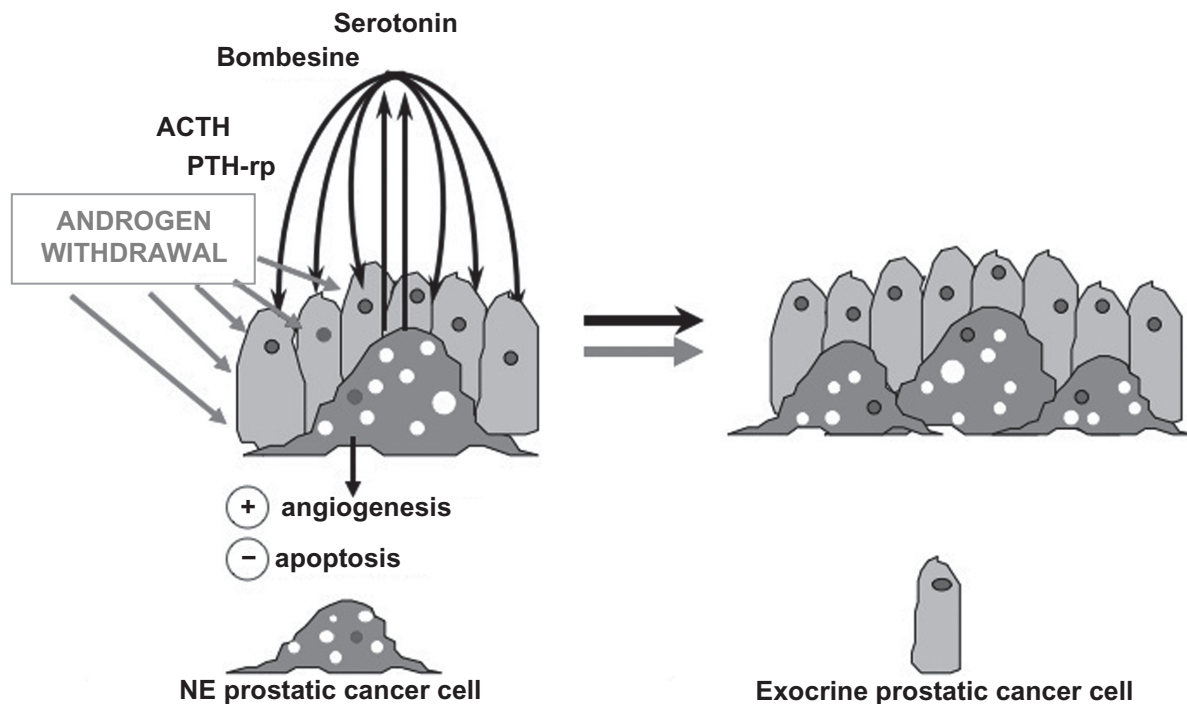


Figure 2 Androgen deprivation therapy stimulates the neuroendocrine differentiation, thus amplifying the negative interaction with the non-neuroendocrine compartment. **Abbreviations:** ACTH, adrenocorticotrophin; PTHrP, parathyroid hormone-related protein; NE, neuroendocrine.

two study arms,⁶⁶ these data cannot exclude that in a patient subset, ie, patients destined to develop a NE phenotype, the administration of finasteride could have been detrimental. In a small randomized placebo controlled neoadjuvant trial, finasteride administration, was in fact able to decrease the apoptotic factor caspase 3,⁶⁷ it would be interesting to know whether this phenomenon was associated or not to a development of a NE phenotype.

Clinical data

The first paper showing the frequent elevation of circulating chromogranin levels in PC patients with castrate resistant metastatic disease was published in 1991 by Kadmon et al.⁶⁸ Twelve out of 25 (48%) patients enrolled had elevated CgA levels. These results were subsequently confirmed by other authors,^{63,70} and CgA is actually considered the best circulating marker for detecting and monitoring the NE differentiation in PC.

Two papers have explored the prognostic role of NE differentiation, assessed by circulating CgA, in PC patients with castrate resistant disease. The first one is a single institution experience,²⁶ the second one refers to a data set of patients enrolled in a multicenter prospective clinical trial of the South West Oncology Group (SWOG).⁷¹ As outlined in Table 1, both studies uniformly showed that elevated CgA levels were significantly correlated with a poor prognosis. It is interesting

to note that in multivariate analysis after adjustment for commonly recognized prognostic factors (such as hemoglobin, albumin, lactate dehydrogenase [LDH], alkaline phosphatase) the hazard ratio (HR) of death from elevated CgA values was superimposable in both studies (close to 1.2).

Eighteen studies have explored the significance of NE differentiation in hormone naïve patients (Tables 2 and 3).^{72–89} Twelve studies enrolled patients with nonmetastatic disease (T1–4, N0 or N1) that were eligible for radical prostatectomy.^{73–76,78–82,86,87,89} In two of these studies one half of patients enrolled received neoadjuvant hormone therapy before surgery.^{73,86} One study enrolled patients with T1–4, N0–1 PC who underwent radiation therapy only.⁸⁵ The remaining five studies were performed on patients with all stages of disease submitted to heterogeneous treatments (two papers),^{72,77} or included metastatic patients who underwent ADT (three papers).^{83,84,88}

NE differentiation was assessed immunohistochemically in tumor specimens from radical prostatectomy in 11 papers,^{72–75,78–82,86,89} in 2 of them this phenotype was also assessed in abdominal lymphnodes.^{74,86} In three papers NE differentiation was assessed on prostate biopsies,^{65,72,77} while in two papers it was assessed on transurethral resection of the prostate (TURP) specimens (in one of them after a pretreatment with hormone therapy).^{77,84} In the remaining two papers, circulating CgA was employed instead of tissue evaluation.^{85,87}

Table 1 Prognostic role of chromogranin A serum levels in castrate resistant prostate cancer

1st Author	No. of patients	Stage of disease	Follow-up time	Median overall survival (univariate analysis)	Overall survival all cause of death: (multivariate analysis)	NE ^a assessment
Berruti 2005 ²⁶	108	Hormone-refractory disease	Median 72 months	Supranormal CgA ^b levels: 10.2 months Normal CgA levels: 22.6 months $P = 0.0002$	Supranormal CgA levels: HR: 1.20 (95% CI ^d 0.99–1.44), $P = 0.05$	Serum CgA levels at the onset of hormone refractory disease
Taplin 2005 ⁷¹	321	Metastatic hormone-refractory disease	Median 35 months	Serum CgA > 12 U/l: 11 months Serum CgA ≤ 12 U/l: 17 months $P = 0.01$ Serum CgA > 9.5 U/l: 12 months Serum CgA < 9.5 U/l: 18 months $P = 0.021$	Serum CgA > 12 U/l: HR 1.214 (95% CI 0.967–1.525), $P = 0.0952$ Serum CgA > 9.5 U/l: HR: 1.326 (95% CI 1.045–1.683), $P = 0.0203$	Serum CgA levels at the onset of hormone refractory disease

Abbreviations: ^aNE, neuroendocrine; ^bCgA, chromogranin A; ^cHR, hazard ratio; ^dCI, confidence interval.

As a whole the prognostic role of NE differentiation, assessed on baseline conditions, have led to controversial results. However these studies suffer from several limitations in terms of statistical power, primary prognostic end-point chosen and patient selection. Most of these studies have recruited a limited number of patients: seven studies, in fact, involved less than 100 patients,^{72,77,78,81,83,84,88} nine studies between 100 and 200 patients,^{73–76,79,80,85,86,89} while only two studies considered more than 200 patients.^{82,87} Due to the low potency of the majority of studies it is not surprising that many of them have led to inconclusive results.

The prognostic end point was time to progression in 10 studies,^{72,73,75,79,80,82,84,85,87,89} while overall survival was considered in only 8 studies.^{74,76,77,78,81,83,86,88} In most studies it was the time of PSA progression that was tested, which is actually considered a questionable end point.⁹⁰ As a matter of fact in one study in which both time to PSA progression and time to clinical disease progression were concomitantly assessed, NE phenotype appeared prognostic in terms of time of clinical progression but not in terms of PSA progression.⁸⁰

Another important issue is patient selection, most studies recruited patients eligible for radical prostatectomy. This patient population, due to low stage and grade, is destined per se to a good prognosis even without treatment and it is therefore not representative of the entire PC population.

These limitations notwithstanding, the prognostic significance of NE phenotype was observed in 10 studies (Table 2),^{80–89} whereas in eight papers NE differentiation failed to be prognostic (Table 3).^{72–79}

If we look at the nonsignificant studies (Table 3), however, the prognostic significance of NE differentiation was found to be truly negative in three studies only. Bostwick et al including 196 patients with node positive carcinomas who underwent radical prostatectomy plus lymphadenectomy, showed superimposable disease-free survival rates and overall survival by comparing patients with immunohistochemical NE differentiation to patients without: HR 0.94 and 1.02, respectively.⁷⁴ In a further study, Nordzij et al examined 90 patients who underwent radical prostatectomy and were followed for 86 months on average. The results showed that the presence of NE features was associated with a similar disease-free and overall survival prospect as compared with the absence of this phenotype.⁷⁸ In contrast to all published studies, Veltri et al showed that the presence of NE differentiation correlated with a tendency toward a better prognosis in terms of disease-free survival that just failed to attain the statistical significance ($P = 0.12$).⁷⁹ In the remaining five papers, NE differentiation was associated with a tendency toward a poorer prognosis that failed to attain statistical significance, probably due to the low potency of these studies.^{72,73,75–77}

In the study by McWilliams et al the prognostic role of NE differentiation attained the statistical significance in univariate but not in multivariate analysis.⁷⁷

The 10 papers in which NE differentiation was found to be prognostic comprised patients submitted to radical prostatectomy for early disease (six papers),^{80–82,86,87,89} locally advanced or metastatic disease addressed to ADT (three papers),^{83,84,88}

Table 2 Studies showing a significant prognostic role of neuroendocrine phenotype

Ist Author	No. of patients	Stage of disease	Therapies	Follow-up time	Disease free survival: (univariate analysis)	Disease free survival: (multivariate analysis)	Overall survival carcinoma specific death (univariate analysis)	Overall survival carcinoma specific death (multivariate analysis)	Overall survival all cause of death (univariate analysis)	NE ^a assessment
Autorino 2007 ⁸⁰	110	pT2: 65 (59%) pT3: 45 (41%) pN+: 13	RP ^b	Median 64 months	HR: 2.08 (95% CI: n.a.), P = 0.03	HR: 2.04 (95% CI: n.a.), P = 0.04	n.a.	n.a.	n.a.	IHC ^c staining for CgA ^g on RP specimens.
Gunja 2008 ⁸²	528	0.75 pT2b: 268 pT3a: 85 (16.10%) pT3b: 64 (12.10%) pT4: 12 (2.30%) pN0: 412 (78.05%) pN1: 38 (7.15%) pNx: 78 (14.80%)	RP	Median 46.4 months	Patients disease free surviving after 5 years: NE pos ^h : 49.1% NE neg ⁱ : 80.6% P < 0.001	HR: 1.89 (95% CI 1.06–3.37) P = 0.032	n.a.	n.a.	n.a.	IHC staining for CgA on RP specimens.
Kamiya 2008 ⁸³	58	AUA Stage D2: 58 (100%)	ADT ^k estramustine dexamethasone.	Median 144 months	n.a.	n.a.	NE pos: 26 months NE neg: > 140 months. P = 0.0379 .	HR: 3.85 (95% CI 1.2–14.3), P = 0.02.	n.a.	IHC staining for CgA on needle biopsy specimens. Pretreatment serum CgA levels.
Krijnen 1997 ⁸⁴	72	All stages	ADT	Median 36 months	Patients disease free surviving after 50 months: NE neg: 66% . NE pos: 40% P = n.a.	Adjustment for Gleason: NED has independent prognostic role for DFS (P < 0.001).	n.a.	n.a.	n.a.	IHC staining for CgA on TURP ^m specimens.
Lilleby 2001 ⁸⁵	161	T1–4 pN0 M0	RDT ⁿ	Median 132 months	NED pos: 41 months NED neg: 80 months P = 0.001	NSE ^o pre-treatment: HR: 2.27 (95% CI 1.37–3.35), P = 0.0014 NSE 3 months post-treatment: HR: 2.52 (95% CI 1.52–4.19), P = 0.0065	n.a.	n.a.	n.a.	Serum levels of NSE e CgA before and 3 months after RDT.

(Continued)

Table 2 (Continued)

Ist Author	No. of patients	Stage of disease	Therapies	Follow-up time	Disease free survival: (univariate analysis)	Disease free survival: (multivariate analysis)	Overall survival carcinoma specific death (univariate analysis)	Overall survival carcinoma specific death (multivariate analysis)	Overall survival all cause of death (univariate analysis)	NE ^a assessment
Quek 2006 ⁸⁶	140	pT2 pN+: 15 (10.70%) pT3 pN+: 100 (71.45%) pT4 pN+: 25 (17.85%)	RP Neoadjuvant RDT/ADT: 45 (32%) Adjuvant RDT/ADT: 107 (76%)	Median 129 months	HR: 2.11 (95% CI 1.04–4.26), P = 0.031.	n.a.	n.a.	n.a.	Primary cancer: HR: 1.89 (95% CI 0.95–3.82), P = 0.063 Lymph nodes: HR: 2.10 (95% CI 1.04–4.28), P = 0.032	IHC staining for CgA on benign tissue, primary prostate cancer and lymph node metastases.
Sciarra 2007 ⁸⁷	264	pT2: 142 (53.8%) pT3: 122 (46.2%)	RP	Median 60 months	Patients disease free surviving after 75 months: CgA < 60 ng/ml: 82% CgA > 60 ng/ml: 50% P < 0.0001 CgA < 90 ng/ml: 78% CgA > 90 ng/ml: 20% P < 0.0001	HR: 1.85 (95% CI 1.24–2.56), P = 0.001	n.a.	n.a.	n.a.	Preoperative serum CgA levels.
Theodorescu 1997 ⁸¹	71	AUA Stages A2 to B2	RP	54% of patients ≥ 120 months	n.a.	n.a.	HR: 1.527 (95% CI 1.021–2.282), P = 0.0353.	Two-variable model (CathD ^p and CgA): HR: 1.463 (95% CI 1.003–2.13), P = 0.055. One-variable model (CgA): HR: 1.498 (95% CI 1.013–2.216), P = 0.043.	HR: 0.741 (95% CI 0.409–1.341), P = 0.3120.	IHC staining for CgA on RP specimens.

Yamada 2006. ⁸⁸	50	AUA stage D2: 50 (100%)	ADT	Median 48.7 months	NE pos: 11 months NE neg: 18 months $P = 0.025$	n.a.	Surviving patients after 5 years: NE pos: 34.1% NE neg: 55.2% $P = 0.37$	n.a.	IHC staining for CgA on needle biopsy specimens.
Weinstein 1996. ⁸⁹	104	T1 or T2	RP	Median 96 months	Patients disease free surviving after 6 years: G.s. ⁴ 5–6 tumors: NED pos: 50% NED neg: 90% $P < 0.008$ All tumors: NED pos: 50% NED neg: 60% $P < 0.036$	n.a.	n.a.	n.a.	IHC staining for CgA on RP specimens.

Abbreviations: ¹NE, neuroendocrine; ²RP, radical prostatectomy; ³HR, hazard ratio; ⁴CI, confidence interval; ⁵n.a., not available; ⁶IHC, immunohistochemical; ⁷CgA, chromogranin A; ⁸pos., positive; ⁹neg., negative; ¹⁰AUA, American Urology Association; ¹¹ADT, androgen deprivation therapy; ¹²NED, neuroendocrine differentiation; ¹³TURP, transurethral resection of the prostate; ¹⁴RDT, radiotherapy; ¹⁵NSE, neuron specific enolase; ¹⁶CathD, cathepsin D; ¹⁷G.s., Gleason score.

and patients who underwent radiation therapy only (one paper).⁸⁵ In six papers the prognostic significance was assessed in terms of time to progression,^{80,82,84,85,87,89} in four papers survival was also considered.^{81,83,86,88} In nine papers NE differentiation was assessed by CgA expression, either at immunohistochemistry or in the general circulation.^{80–84,86–89} In one paper both circulating NSE and CgA were evaluated.⁸⁵ Noteworthy, in this latter study, elevated serum NSE but not elevated serum CgA showed a prognostic significance.

Our personal experience

We have investigated the prognostic role of NE differentiation assessed on PC biopsies in 414 PC patients consecutively treated and followed at the PC unit of the Azienda Ospedaliero-Universitaria San Luigi Gonzaga, Orbassano, Italy. The median follow-up was 85 months. Two hundred and fourteen patients received ADT within the first 2 months of diagnosis in addition to local regional therapies (radiation therapy or radical prostatectomy) if indicated, 200 patients were submitted to local regional therapies alone. Since ADT stimulates NE differentiation, CgA plasma levels were determined at baseline condition and subsequently every year for 2 years in the patient subset immediately addressed to such a therapy. The results of this study were published in two different papers and are summarized in Table 4.^{91,92}

In multivariate analysis, after adjustment for age, stage, serum PSA, Gleason score and treatment administered (hormone therapy, radical prostatectomy, radiation therapy), NE differentiation assessed on baseline PC biopsies was prognostic in overall population in terms of overall survival (HR 1.56, 95% Confidence Interval [CI] 1.12–2.17, $P = 0.009$) but not in terms of PSA progression (HR 1.25, 95% CI 0.96–1.62, $P = 0.10$). However, dividing patients according to whether they received early androgen deprivation or not, the prognostic significance of NE differentiation, either in terms of disease-free survival or overall survival, appeared to be confined to the first patient subset. A significant prognostic interaction between ADT and tissue CgA expression was found in multivariate analysis in terms of time to progression, but not in terms of overall survival. It should be noted, however, that the majority of patients initially not submitted to ADT received this treatment after disease recurrence and this represents a confounder that may have accounted for the observed no interaction in terms of survival.

In the patient subset immediately addressed to androgen deprivation, elevated serum CgA was significantly associated with a poor prognosis in terms of progression-free survival and overall survival. These data paralleled the prognostic

Table 3 Studies failing to show a significant prognostic role of neuroendocrine phenotype

Ist Author	No. of patients	Stage of disease	Therapies	Follow-up time	Disease free survival (univariate analysis)	Disease free survival (multivariate analysis)	Overall survival specific cancer death (univariate analysis)	Overall survival all cause of death (univariate analysis)	Overall survival cause of death (multivariate analysis)	NE ^a assessment
Abrahamsson 1998 ⁷²	87	AJA ^b stages A: 1 (1.1%) B: 27 (31.2%) C: 50 (57.4%) D: 9 (10.3%)	RP ^c	Median 50 months	Proportion disease progression: NE pos ^d : 43% NE neg ^e : 33% P = 0.368	n.a. ^f	n.a.	n.a.	n.a.	IHC ^g staining for CgA ^h on RP specimens.
Ahlgren 2000 ⁷³	103	T1b-1c: 39 (37.9%) T2-3: 64 (62.1%)	Neoadjuvant ADT+ RP: 50 (48.5%). RP: 53 (51.5%).	Median 39 months	Disease free patients after 25 months Neoadjuvant group: NE pos: 69% NE neg: 92% surgery only group: NE pos: 67% NE neg: 79% P = 0.03	Neoadjuvant group: HR: 1.4 (95% CI) ^k 0.4–4.5), P = 0.54. Surgery only group: HR: 1.5 (95% CI 0.7–3.2), P = 0.29.	n.a.	n.a.	n.a.	IHC staining for CgA on RP specimens.
Bostwick 2002 ⁷⁴	196	pN+: 196 (100%)	RP Adjuvant ADT: 94%.	Median 80 months	HR: 0.94 (95% CI 0.84–0.05), P > 0.1.	n.a.	HR: 1.01 (95% CI 0.96–0.07), P > 0.1.	HR: 1.02 (95% CI 0.99–1.05), P > 0.1.	n.a.	IHC staining for CgA on primary prostate cancer and lymph node metastases.
Bubendorf 1996 ⁷⁵	137	pT1: 4 (2.9%) pT2: 43 (31.4%) pT3: 90 (65.7%) pN+: 34 (24.81%)	RP: 87 (63.5%) RP+RDT: 44 (32.1%) RP + orchiectomy: 6 (4.4%)	Median 64 months	Disease free patients after 8 years: NE pos: 59% NE neg: 62% P = n.s. ^m	n.a.	n.a.	n.a.	n.a.	IHC staining for CgA on RP specimens.
Casella 1998 ⁷⁶	105	≥pT1c: 105 (100%)	RP: 15 (14%) RDT: 14 (13%) TURP ⁿ : 47 (45%) Orchiectomy: 54 (51%)	Median 44 months	n.a.	n.a.	Median survival: NE neg: 68 months NE pos: 60 months P = n.s.	n.a.	n.a.	IHC staining for CgA on needle biopsy specimens.

Mc William 1997 ⁷⁷	92	T1 a + b M0: 30 (32.6%) T2a M0: 15 (16.30%) T2b M0: 19 (20.65%) T3 M0: 9 (9.8%) M1: 19 (20.65%)	n.a.	From 84 months to 156 months	n.a.	n.a.	Surviving patients after 96 months: NE pos: 40% NE neg: 72% P = 0.036	Prognostic role of NE pos. not confirmed in multivariate analysis: P = 0.31	IHC staining for CgA on TURP specimens.
Noordzij 1995 ⁷⁸	90	RP pT2: 22 (24.4%) pT3: 66 (73.4%) pT4: 2 (2.2%) pN1: 5 (5.5%) pN2: 2 (2.2%)	RP	Median 86 months	n.a.	Disease free patients after 96 months NE pos: 54% NE neg: 58% P = 0.81	Surviving patients after 96 months: NE pos: 76% NE neg: 80% P = 0.84	n.a.	IHC staining for CgA on RP specimens.
Veltri 2008 ⁷⁹	105	T2a: 58 (55.2%) T2b/c: 47 (44.8%) N0: 105 (100%)	RP	Median 207 months	n.a.	Continuous variable: HR: 0.89 (95% CI 0.50–1.56), P = 0.674 Dichotomized variable: HR: 0.45 (95% CI 0.16–1.24), P = 0.122	n.a.	n.a.	IHC staining for CgA on RP specimens.

Abbreviations: ^aNE, neuroendocrine; ^bAUA, American Urology Association; ^cRP, radical prostatectomy; ^dpos, positive; ^eneg, negative; ^fn.a., not available; ^gIHC, immunohistochemical; ^hCgA, chromogranin A; ⁱADT, androgen deprivation therapy; ^jHR, hazard ratio; ^kCI, confidence interval; ^lRD, radiotherapy; ^mn.s., not significant; ⁿTURP, transurethral resection of the prostate.

Table 4 Our experience on the prognostic role of neuroendocrine phenotype

Ist Author	No. of patients	Stage of disease	Therapies	Follow-up time	Disease free survival: HR ^a (95% CI) ^b (univariate analysis)	Disease free survival: HR (95% CI) (multivariate analysis)	Overall survival all cause of death: HR (95% CI) (univariate analysis)	Overall survival all cause of death: HR (95% CI) (multivariate analysis)	NE ^c assessment
Berruti 2007 ⁹¹	211 for IHC ^d evaluation 175 for serum CgA ^e assessment	Stage T1-2: 85/205 (41.5%) Stage T3-4: 71/205 (34.6%) Stage M1: 49/205 (23.9%) Missing 6	RP ^f : 48/211 (22.7%) RDT ^g : 89/211 (42.2%) ADT ^h alone: 74/211 (35.1%)	Median 55 months	IHC CgA pos < 30%: HR: 2.0 (1.3-3.1), P = 0.001 IHC CgA pos > 30%: HR: 6.0 (2.7-12.9), P = 0.000	IHC CgA pos < 30%: HR: 1.7 (1.0-2.8), P = 0.03 IHC CgA pos > 30%: HR: 3.9 (1.7-9.0), P = 0.001	IHC CgA pos < 30%: HR: 1.9 (1.2-3.0), P = 0.0063 IHC CgA pos > 30%: HR: 5.7 (2.3-13.9), P = 0.000 Elevated serum CgA at baseline: HR: 2.4 (1.4-3.9), P = 0.000 at year 1: HR: 4.8 (2.9-7.9), P = 0.000 at year 2: HR: 2.4 (1.2-4.7), P = 0.008	IHC CgA pos < 30%: HR: 1.5 (0.9-2.6), P = 0.13 IHC CgA pos > 30%: HR: 3.4 (1.3-8.9), P = 0.01	IHC staining for CgA on needle biopsy specimens Serum CgA levels at diagnosis and after 1 year and 2 years
Berruti 2009 ⁹²	200	Organ confined/locally advanced disease	No therapy: 2/200 (1%) RP: 102/200 (51%) RDT: 93/200 (46.5%) RP + RDT: 3/200 (1.5%)	Median 85 months	HR: 0.87 (0.60-1.26), P = 0.47	HR: 0.87 (0.58-1.30), P = 0.49	HR 1.28 (0.79-1.28), P = 0.32	HR 1.49 (0.83-2.51), P = 0.19	IHC staining for CgA on needle biopsy specimens

Abbreviations: ^aHR, hazard ratio; ^bCI, confidence interval; ^cNE, neuroendocrine; ^dIHC, immunohistochemical; ^eCgA, chromogranin A; ^fRP, radical prostatectomy; ^gRDT, radiotherapy; ^hADT, androgen deprivation therapy; ⁱpos, positive.

results obtained with the immunohistochemical CgA evaluation.

As expected, the proportion of patients with elevated serum CgA increased over time, being 20% at baseline, 25% after one year and 35% after two years.

Interestingly, the prognostic significance of elevated serum CgA levels was maintained at every time point. Since the patients with increased CgA at each point were not the same ones showing increased CgA at baseline, these data suggest that increasing CgA during ADT may be a signal of changed tumor biology toward a greater aggressiveness and resistance to endocrine therapy.⁹¹

NE differentiation as a target for specific molecular target agents

Since the postulated mechanisms by which NE differentiation enhances the aggressiveness of PC is the paracrine stimulation of the exocrine component of the tumor, there is a rationale for the clinical use of somatostatin analogs that are notoriously able to inhibit the synthesis of neusecretory products.

Few data exist about the selected antiproliferative effect of somatostatin in preclinical model systems. An *in vitro* study has shown that somatostatin is able to reduce the proliferation of LNCaP cells.⁹³ In a further study adjuvant therapy with lanreotide was found effective in slowing cancer growth in castrated Copenhagen rats bearing Dunning R-3327-H prostate tumors.⁹⁴

In an elegant *in vitro* study recently published, Ruscica and coworkers investigated the effects of lanreotide and new mono- and bi-specific somatostatin agonists on proliferation and secretory pattern of the insulin growth factor (IGF) system in LNCaP cells, a model of androgen-dependent PC.⁹⁵ They found that all these somatostatin analogs have an antiproliferative effect against LNCaP PC cells. This antiproliferative activity, mediated by an induction of p27 and p21 cell-cycle inhibitors and a down-regulation of protein expression of cyclin E, was counteracted by the incubation with a somatostatin receptor subtype-2 (sst2) antagonist. These data suggest a role for the sst2 receptor in mediating this effect. All somatostatin analogs caused a significant reduction in IGF-I and IGF-II secretion. Noteworthy, the administration of exogenous IGF-I counteracted the inhibitory effect on cell proliferation of these compounds. These data are consistent with a role of the IGF system in stimulating the PC growth and the importance of IGF-I inhibition as an adjunctive mechanism of antineoplastic effect of somatostatin analogs.

These preclinical data notwithstanding, somatostatin analogues have shown a modest activity or no activity in PC patients with hormone refractory disease in few uncontrolled phase II trials.^{96–100} Since in these studies the primary aim was reduction in PSA serum levels or objective clinical response and not the time to progression, they cannot exclude a possible effect of these drugs in terms of prolongation of disease stabilization. Somatostatin analogs are long since recognized as the best antisecretory treatment of advanced well differentiated NE carcinomas. The antineoplastic effects of these drugs in these tumors, however, have been demonstrated only recently. In a randomized, placebo controlled, phase 2 study involving metastatic well-differentiated mid-gut carcinoma, in fact, octreotide administration was able to significantly prolong the time to progression despite a no-effect observed in terms of disease response.¹⁰¹

We conducted a small single institution prospective study in which PC patients with hormone refractory disease and NE differentiation received the somatostatin analog lanreotide. In this study lanreotide was able to significantly decrease the circulating levels of chromogranin A.¹⁰² Of course the changes of chromogranin A could not be considered a surrogate parameter of treatment efficacy, but these data provide the rationale of testing the efficacy of somatostatin analogs in prolonging the time to progression in a prospective randomized clinical trial in this patient subset.

Due to the poor expression of sst2 in patients with hormone naïve disease,¹⁰³ no studies have tested the somatostatin analogs in addition to ADT in these patients. The availability in the near future of multireceptor ligand compounds with high affinity to somatostatin receptor subtypes sst1, sst2, sst3, and sst5 (such as pasireotide [SOM230]),¹⁰⁴ will stimulate studies also in this subset.

Finally, as mentioned previously, the NE phenotype in PC patients is associated with a greater expression of the PI3K/AKT/mTOR pathway, providing a rationale for the use of mammalian target of rapamycin (mTOR) inhibitors.³² Rapamycin analogues are currently tested in PC patients with the aim to overcome the endocrine resistance. It would be interesting to know whether they are more active in the patient subset with NE differentiation.

Conclusion

The potential negative prognostic role of the coexistence of NE differentiation in the context of a predominant exocrine component of prostate adenocarcinoma is suggested by several preclinical observations and substantially confirmed by clinical data. The majority of clinical studies have, in fact,

shown a significant correlation between NE differentiation and disease prognosis, and several studies have shown a tendency (not significant) toward this association. NE differentiation is not a stable phenotype, being stimulated by a variety of agents including commonly employed antineoplastic therapies. The proportion of PC patients with NE features, therefore, is destined to increase during the natural history of the disease.

The NE phenotype can be a pure prognostic parameter or influence the prognosis by favoring the onset of a castrate resistance status. It is actually not known which is the prevailing mechanism. Our personal experience is in favor of the second mechanism since the prognostic significance of this phenomenon in our series of patients was mainly confined to the patient subset who received ADT shortly after diagnosis as opposed to those who did not.

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Disclosure

The authors report no conflicts of interest in this work.

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