Submitted: 02 March, 2021 Accepted: 22 March, 2021 Published: 30 September, 2021 DOI:10.31083/jomh.2021.040

JOMH Journal of Men's Health

Mini-Review

Mechanisms of castration resistant prostate cancer formation and progression through neuroendocrine differentiation

Xueping Ma[1], Xu Jiang[1], Xuezhen Yang[1,*]

[1]Department of Urology, the Second Affiliated Hospital of Bengbu Medical College, 233020 Bengbu, Anhui, China

***Correspondence:** engineyang@sina.com (Xuezhen Yang)

Abstract

Normal prostate tissues consist mainly of epithelial cells, including secretory epithelial cells, basal cells, and neuroendocrine cells, and of mesenchymal cells, including smooth muscle cells and fibroblasts. The mechanisms leading to castration resistant prostate cancer (CRPC) are complex and diverse, but most involve neuroendocrine differentiation. In fact, during the development of prostate cancer, some of the tumor cells transform into neuroendocrine-like cells. This transition is a main underlying mechanism of CRPC formation.

Keywords

Prostate cancer; Neuroendocrine differentiation; Castration resistance prostate cancer

Castration resistant prostate cancer (CRPC) refers to the stage of prostate cancer that follows initial treatment by androgen deprivation. It should be differentiated from metastatic hormone-sensitive prostate cancer (mHSPC). Metastatic CRPC (mCRPC) corresponds to the metastatic stage of hormone-insensitive prostate cancer. In recent years, the diagnosis and treatment for prostate cancer have gradually improved, but the survival rate of the patients did not significantly increase. CRPC is the final cause of death for patients with advanced prostate cancer, and it has remained a difficult challenge for urologists for many years [1]. Currently, it is known that neuroendocrine differentiation (NED) and neuroendocrine prostate cancer (NEPC) are a main cause of emergence of CRPC, which mostly occurs after long-term castration treatments. NEPC mainly manifests by a decreased expression of prostate-specific markers such as androgen receptor (AR) and prostate-specific antigen (PSA), and increased expression of neuro-related markers, such as pheochromogranin A, synaptophysin, and neuron-specific enolase. Prostate cancer patients with neuroendocrine (NE) characteristics have poor response to chemotherapy and poor prognosis, most of them facing a survival prospect of less than one year [2].

In early studies, there was much debate about the status and significance of NEPC and NED in prostate cancer [3]. Since then, with the help of emerging technologies such as gene sequencing and genetically engineered mice, it has become increasingly clear that there are significant differences in gene expression profiles and biological properties between NEPC and CRPC. Therefore, specific therapies that target patients with NE characteristics are needed in order to improve the prognosis of patients with advanced prostate cancer [4]. To date, the mechanisms underlying NED during prostate cancer is unclear, and there are no effective drugs able to block this process. In addition, there is no real specific drug for NEPC treatment. The survival time of patients diagnosed at early stages of prostate cancer after effective treatment is significantly longer than that of patients diagnosed at advanced stages. For prostate cancer, early diagnosis is a unique opportunity to take the life of the patients out the hands of prostate cancer. Currently, there are many hypotheses regarding the formation and regulation of NEPC and NED. In this review, we report research progresses from current literature to provide a more comprehensive understanding of NEPC and NED.

1. When does NED occur during treatment of prostate cancer?

According to the research of Yang at Columbia University *et al.* [5], during treatment of prostate cancer most patients experience NED upon androgen deprivation. However, at this stage, prostate cancer is not a CRPC. The influence of NED gradually increases before turning into CRPC eventually.

A large number of clinical and experimental studies have confirmed that NED plays a key role in the occurrence and development of prostate cancer. Therefore, further research focusing on understanding the etiology of NED, how NED promotes drug resistance, which signaling pathways can prevent or reverse NED, and what theoretical basis of drugs or biological agents should be considered towards targeted therapy, will warrant broad prospects to improve the survival rate and quality of life of prostate cancer patients.

2. Genetic alterations

2.1 Inactivation of tumor suppressor genes

Mutations or deletions in tumor suppressor genes have been a main focus of research and discussions in the field of cancer. In prostate cancer-related NED, the genes retinoblastoma (*RB*), tumor protein 53 (*TP53*), and phosphatase and tension homology gene deleted on chromosome ten (*PTEN*) received most of the attention. Clinical studies revealed that oncogenic alterations affecting these genes are more prevalent in prostate cancer patients with NE features than in CRPC patients, with 70% of NED cases presenting with *RB* gene deletions, 66.7% with *TP53* mutations or deletions, and 53.3% with *TP53* and *RB1* co-deletions [4, 6]. *RB* gene deletion is present in 90% of prostate small-cell neuroendocrine carcinoma (SCNC), while in other types of prostate cancer such as high-grade adenocarcinoma or metastatic CRPC this frequency is less than 20% [7]. It follows that inactivation of tumor suppressor genes is highly correlated with prostate cancer-related NED. Meanwhile, the importance of tumor suppressor genes has been demonstrated in basic research. Studies of the prostate cancer pathology have shown that inactivation of *PTEN* is clearly associated with elevated expression of the NED marker chromogranin A [8]. Selective knockout of *Rb* and *Trp53* genes in mouse spontaneously generates hypo-differentiated prostate adenocarcinoma with NE characteristics. A common transgenic adenocarcinoma of mouse prostate (TRAMP) developing spontaneous NEPC was also used in a similar setup [9]. Recent studies have shown that genetically engineered mice with co-deletion of *Trp53* and *Ptem* (NPp53 mouse) develop abiraterone-resistant prostate cancers with behaviors highly consistent with those of human CRPC with NE characteristics [10]. Current mainstream research suggests that the inactivation of tumor suppressor genes is not an isolated event, but in fact often coexists with many other the biological events such as those mentioned below, and serves as a prerequisite for the induction of NED by other molecules such as serine/arginine repetitive matrix 4 (SRRM4) [11].

2.2 Activation of proto-oncogenes

Activation of proto-oncogenes can promote a series of changes in the biological behavior of tumors, such as proliferation, invasiveness, and migration. The members of the Myc family of protooncogenes have attracted much attention due to their extensive cancer-promoting ability in a variety of tumors. Different from *MYCC*, which is expressed in a variety of tumors, *MYCN* is only highly expressed in a few neurological tumors such as retinoblastoma, and in small-cell lung cancer, indicating that this gene is closely related to neurological phenotypes [12, 13]. *MYCN* has also been shown to play an important role for NED in prostate cancer. Beltran *et al.* [14] found that *MYCN* gene amplification or overexpression was present in 40% of NEPC, while this proportion was only 5% in ordinary prostate cancers. Overexpression of *MYCN* in normal prostate cell lines and basal cells of normal prostate can lead to NE phenotype [15]. Later, Dardenne *et al.* [16] found that MYCN protein could interact with the product of enhancer of zeste homolog 2 (*EZH2*), a homolog of the histone methyltransferase zeste gene that binds the androgen receptor (*AR*) gene enhancer. By promoting the expression of a series of genes related to cell stemness and cell migration, and inhibiting AR signaling pathway, EZH2 allows prostate cancer to survive better under anti-androgen therapy and suppressed AR signaling. This mechanism explains the resistance of NEPC to anti-androgen therapy. In addition, through gene analysis of circulating tumor cells, Gupta *et al.* [17] found that tumor cells from patients with high MYCN expression would differentiate into AR-independent NE-like cells, suggesting that this gene has a high predictive value for castration-resistance and NED in patients.

2.3 Gene fusions

As early as 2005, Tomlins *et al.* [18] reported the fusion between E26 transformation-specific (*ETS*)-related gene (*ERG*) and the transmembrane protease serine 2 (*TMPRSS2*) gene in prostate cancer, and they proposed that this genetic mutation was directly related to the occurrence of prostate cancer. However, it was later found that this gene fusion was not directly responsible for the emergence of prostate cancer, but it was associated with its progression [19]. Clinicopathological studies have confirmed the presence of *TMPRSS2:ERG* gene fusion in 44% of the NEPC. Furthermore, the immunohistochemical study of mixed tumors indicated that NE lesions highly coincided with *TMPRSS2:ERG*-positive areas, and that NED was only localized in areas where ERG protein was deficient [14]. Further studies showed that the presence of *TMPRSS2:ERG* gene fusion promotes the occurrence of NED in prostate cancer patients after anti-androgen therapy [20]. Restoring ERG signaling in *TMPRSS2:ERG*-bearing cells reverses their NED to the parental phenotype [21]. This suggests that the presence of the *TMPRSS2:ERG* gene fusion may be a prerequisite for the induction of NED in prostate cancer during anti-androgen therapy. In addition, *TMPRSS2:ERG* gene fusion has long been considered a gene imprinting signature of prostate cancer, and its discovery in

NEPC largely corroborates that NED is a main mechanism of CRPC formation [14].

3. Gene expression changes

3.1 Transcriptional regulation

In the course of prostate cancer development, from the earliest intraepithelial neoplasia to CRPC or even NEPC, the gene expression profile of the cells undergoes significant changes involving crucial roles of transcription factors such as Foxhead box (FOX) protein family. FOXA1 is a pioneer factor that controls the expression of AR-related genes and intervenes during the whole process of tumorigenesis and cancer development [22]. FOXA2 is an important regulatory molecule in NEPC, although it has received less attention than FOXA1 in prostate cancer. It was found that the positive expression rate of FOXA2 in prostate SCNC specimens is 100% [23]. FOXA2 up-regulation was also observed in transgenic animal models characterized by NEPC [15]. In terms of molecular mechanisms, FOXA2 activates androgen-related genes independently of AR and androgen [24]. Together with the hypoxia-inducible factor 1α (HIF-1α), FOXA2 promotes the expression of a series of key genes that determine the acquisition of a NE phenotype [25]. These results suggest that FOXA2 can promote NED. To further confirm the importance of FOXA2 for NED in prostate cancer, TRAMP mice, which spontaneously develop NEPC, had the FOXA2 gene knocked out. However, Foxa2-deficiency did not prevent NEPC development, leading the investigators to suggest that other important molecules may control NED in the absence of FOXA2 [26].

3.2 Post-transcriptional regulation

Alternative splicing is an important biological mechanism involved in the post-transcriptional regulation of progressive prostate cancer. It has a clear association with tumor aggressivity, drug resistance and other adverse characteristics, in particular through the generation of variable transcripts of *AR* [27, 28]. The successive studies in the field uncovered an increasing number of post-transcriptional modifications linked to advanced prostate cancer. Lapuk *et al.* [29] first found that the level of the RE1 silencing transcription factor (REST) was significantly downregulated in prostate cancer with NED. The expression levels of various NED markers increased after *REST* gene knockout in the prostate cancer cell line LNCaP. Similarly, in prostate cancer cells, REST is inhibited during interleukin 6 (IL-6)-induced NED development, and overexpression of REST can block IL-6-induced NED development [30]. Hypoxic environments can also down-regulate REST level through microRNA (miRNA) and induce NED in prostate cancer cell lines [31]. In prostate cancer, REST cannot operate without another important molecule, SRRM4, which is a neuro-specific RNA splicing factor. The loss of SRRM4 can affect important processes such as neurite growth and cerebral cortex differentiation [32]. Studies on clinical samples with CRPC metastases have shown that the expression of SRRM4 is closely related to the acquisition of a NE phenotype. SRRM4 can inhibit REST mRNA by changing its sequence and promotes the development of NED in prostate cancer [33]. Recent studies have shown that the introduction of SRRM4 in LNCaP cells can induce the expression of NE markers and change the cell morphology. In a model of SRRM4-driven NEPC, there are both AR/PSA positive and AR/PSA negative cell subsets, and SRRM4 is only expressed in the AR/PSA negative cells [11]. This suggests that SRRM4 is an important molecule for the transition from CRPC to NEPC.

4. Tumor microenvironment

In addition to alterations in the tumor cells themselves, the surrounding microenvironment—formed by the tissues, immune cells, blood vessels, and extracellular matrix—also plays an influential role in the biological behavior of the prostate cancer. For instance, tumor-associated macrophages and bone marrow-derived suppressor cells can promote prostate cancer progression by secreting cytokines or exosomes, among other factors [34]. IL-6 is an important pleiotropic cytokine in tumor microenvironments. It can mediate immune response and regulate growth, differentiation, survival, and other important physiological processes of normal and tumor cells. As early as 1990, some scholars confirmed that prostate cancer cell lines have IL-6 receptors and secrete IL-6, suggesting an important role of this cytokine in human prostate cancer [35]. Wang *et al.* [36] used IL-6 stimulation to induce permanent NED in LNCaP cells, which constitutes one of the most typical experiments proving a correlation between IL-6 and NEPC. In addition, enzalutamine, an anti-androgen agent, induces NED and promotes the aggregation and activation of tumor-associated macrophages that in turn, secrete IL-6 and promote further aggravation of NED in prostate cancer [37]. Although an increasing number of studies have focused on IL-6 as a powerful cytokine, the specific relationship between IL-6 and NED in prostate cancer is still not fully understood. Drugs targeting the IL-6 signaling pathway have shown modest effects in clinical trials, indicating that the relationship between IL-6 and prostate cancer, especially NEPC, need further studies [38].

Tumor cells live in a complex microenvironment constituted of diverse components, which can inhibit or promote their survival and growth. In fact, the progression of prostate cancer to CRPC corresponds to the transition from an endocrine disease to a paracrine/autocrine disease. Paracrine/autocrine androgen synthesis is an important mechanism of androgen resistance in CRPC. In prostate cancer tissue, including at low-grade, the level of active androgens such as DHT and other growth factors support cell growth. During cancer progression, the prostatic tissue loses its paracrine dependence and switches to an autocrine pathway, where tumor cells produce many factors, including androgens, to support their own survival and growth.

5. Importance of targeted treatment for NEPC and inhibitory mechanisms of prevention

Over the last several years, important advances have been made in the identification and characterization of clinical and pathological CRPC variants. NEPC is one of the most aggressive subtypes; greater knowledge of the disease biology is necessary to develop new treatments and biomarkers to manage this aggressive variant. Drugs that are able to prevent NEPC are still at the development stage.

6. Summary

Yang *et al.* [5] were among the first scholars in the world to study NED in prostate cancer. Since then, not much research has been done in this area. The emergence and progression of NED and NEPC involve complex regulatory networks, which require a combination of molecular alterations within the tumor and in its environment. In addition to the key targets mentioned above, many others were identified in studies that have not been included due to space limitation. From our summary, it appears that the mainstream literature supports the hypothesis that NEPC differentiates from typical prostate cancer cells under various extra-tumoral pressures [4, 6–11]. This hypothesis also suggests that the starting point of NED in prostate cancer may be much earlier than previously thought. A population of prostate cancer cells with NED potential may already exist while the cancer is still at a stage of low malignancy. These NED precursors are likely to be the cause of poor outcomes in progressive prostate cancer. Thus, besides finding treatments for prostate cancers that have developed into NEPC, future studies should focus more on interventions preventing the development of significant NED features in order to improve patients' prognoses.

Author contributions

XPM: Literature review and arrangement. XJ: Literature review. XZY: Writing and revising the manuscript.

Ethics approval and consent to participate

All of the analyses were based on previously published studies; therefore, no ethical approval or patient consent was required.

Acknowledgment

We would like to express our gratitude to all of the people who helped during the writing of this manuscript, and to the peer reviewers for their constructive opinion and suggestions.

Funding

The study was funded by the Projects of Provincial Educational Department, Anhui Province, China (KJ2019A0373), and Bengbu Medical College, Anhui Province, China (BYKC201912).

Conflict of interest

The authors declare no conflict of interest.

References

[1] US Preventive Services Task Force, Grossman DC, Curry SJ, Owens DK, Bibbins-Domingo K, Caughey AB, *et al.* Screening for prostate cancer: US preventive services task force recommendation statement. The Journal of the American Medical Association. 2018; 319: 1901–1913.

[2] Zhang Y, Zheng D, Zhou T, Song H, Hulsurkar M, Su N, *et al.* Androgen deprivation promotes neuroendocrine differentiation and angiogenesis through CREB-EZH2-TSP1 pathway in prostate cancers. Nature Communications. 2018; 9: 4080.

[3] Ather MH, Abbas F. Prognostic significance of neuroendocrine differentiation in prostate cancer. European Urology. 2000; 38: 535–542.

[4] Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, *et al.* Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nature Medicine. 2016; 22: 298–305.

[5] Yang X, Chen MW, Terry S, Vacherot F, Chopin DK, Bemis DL, *et al.* A human- and male-specific protocadherin that acts through the wnt signaling pathway to induce neuroendocrine transdifferentiation of prostate cancer cells. Cancer Research. 2005; 65: 5263–5271.

[6] Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, *et al.* Integrative clinical genomics of advanced prostate cancer. Cell. 2015; 161: 1215–1228.

[7] Tan H, Sood A, Rahimi HA, Wang W, Gupta N, Hicks J, *et al.* Rb loss is characteristic of prostatic small cell neuroendocrine carcinoma. Clinical Cancer Research. 2014; 20: 890–903.

[8] Ham WS, Cho NH, Kim WT, Ju HJ, Lee JS, Choi YD. Pathological effects of prostate cancer correlate with neuroendocrine differentiation and PTEN expression after bicalutamide monotherapy. Journal of Urology. 2009; 182: 1378–1384.

[9] Berman-Booty LD, Knudsen KE. Models of neuroendocrine prostate cancer. Endocrine-Related Cancer. 2015; 22: R33–R49.

[10] Zou M, Toivanen R, Mitrofanova A, Floch N, Hayati S, Sun Y, *et al.* Transdifferentiation as a mechanism of treatment resistance in a mouse model of castration-resistant prostate cancer. Cancer Discovery. 2017; 7: 736–749.

[11] Li Y, Donmez N, Sahinalp C, Xie N, Wang Y, Xue H, *et al.* SRRM4 drives neuroendocrine transdifferentiation of prostate adenocarcinoma under androgen receptor pathway inhibition. European Urology. 2018; 71: 68–78.

[12] Schwab M, Varmus HE, Bishop JM, Grzeschik KH, Naylor SL, Sakaguchi AY, *et al.* Chromosome localization in normal human cells and neuroblastomas of a gene related to c-myc. Nature. 1984; 308: 288–291.

[13] Rickman DS, Schulte JH, Eilers M. The expanding world of N-MYC-driven tumors. Cancer Discovery. 2019; 8: 150–163.

[14] Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, *et al.* Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. Cancer Discovery. 2011; 1: 487–495.

[15] Lee JK, Phillips JW, Smith BA, Park JW, Stoyanova T, McCaffrey EF, *et al.* N-Myc drives neuroendocrine prostate cancer initiated from human prostate epithelial cells. Cancer Cell. 2016; 29: 536–547.

[16] Dardenne E, Beltran H, Benelli M, Gayvert K, Berger A, Puca L, *et al.* N-Myc induces an EZH2-mediated transcriptional program driving neuroendocrine prostate cancer. Cancer Cell. 2016; 30: 563–577.

[17] Gupta S, Li J, Kemeny G, Bitting RL, Beaver J, Somarelli JA, *et al.* Whole genomic copy number alterations in circulating tumor cells from men with abiraterone or enzalutamide-resistant metastatic

castration-resistant prostate cancer. Clinical Cancer Research. 2017; 23: 1346–1357.

[18] Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun X, *et al.* Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science. 2005; 310: 644–648.

[19] Carver BS, Tran J, Chen Z, Carracedo-Perez A, Alimonti A, Nardella C, *et al.* ETS rearrangements and prostate cancer initiation. Nature. 2009; 457: E1–E1.

[20] Volante M, Tota D, Giorcelli J, Bollito E, Napoli F, Vatrano S, *et al.* Androgen deprivation modulates gene expression profile along prostate cancer progression. Human Pathology. 2016; 56: 81–88.

[21] Mounir Z, Lin F, Lin VG, Korn JM, Yu Y, Valdez R, *et al.* TMPRSS2:ERG blocks neuroendocrine and luminal cell differentiation to maintain prostate cancer proliferation. Oncogene. 2015; 34: 3815–3825.

[22] Jung S, Shin S, Kim MS, Baek I, Lee JY, Lee SH, *et al.* Genetic progression of high grade prostatic intraepithelial neoplasia to prostate cancer. European Urology. 2016; 69: 823–830.

[23] Park JW, Lee JK, Witte ON, Huang J. FOXA2 is a sensitive and specific marker for small cell neuroendocrine carcinoma of the prostate. Modern Pathology. 2017; 30: 1262–1272.

[24] Mirosevich J, Gao N, Gupta A, Shappell SB, Jove R, Matusik RJ. Expression and role of Foxa proteins in prostate cancer. the Prostate. 2006; 66: 1013–1028.

[25] Qi J, Nakayama K, Cardiff RD, Borowsky AD, Kaul K, Williams R, *et al.* Siah2-dependent concerted activity of HIF and FoxA2 regulates formation of neuroendocrine phenotype and neuroendocrine prostate tumors. Cancer Cell. 2010; 18: 23–38.

[26] Gupta A, Yu X, Case T, Paul M, Shen MM, Kaestner KH, *et al.* Mash1 expression is induced in neuroendocrine prostate cancer upon the loss of Foxa2. Prostate. 2013; 73: 582–589.

[27] Kallio HML, Hieta R, Latonen L, Brofeldt A, Annala M, Kivinummi K, *et al.* Constitutively active androgen receptor splice variants AR-V3, AR-V7 and AR-V9 are co-expressed in castration-resistant prostate cancer metastases. British Journal of Cancer. 2018; 119: 347–356.

[28] Magani F, Peacock SO, Rice MA, Martinez MJ, Greene AM, Magani PS, *et al.* Targeting AR variant-coactivator interactions to exploit prostate cancer vulnerabilities. Molecular Cancer Research. 2017; 15: 1469–1480.

[29] Lapuk AV, Wu C, Wyatt AW, McPherson A, McConeghy BJ, Brahmbhatt S, *et al.* From sequence to molecular pathology, and a mechanism driving the neuroendocrine phenotype in prostate cancer. Journal of Pathology. 2012; 227: 286–297.

[30] Zhu Y, Liu C, Cui Y, Nadiminty N, Lou W, Gao AC. Interleukin-6 induces neuroendocrine differentiation (NED) through suppression of re-1 silencing transcription factor (REST). Prostate. 2014; 74: 1086–1094.

[31] Liang H, Studach L, Hullinger RL, Xie J, Andrisani OM. Down-regulation of re-1 silencing transcription factor (REST) in advanced prostate cancer by hypoxia-induced miR-106b 25. Experimental Cell Research. 2014; 320: 188–199.

[32] Quesnel-Vallières M, Irimia M, Cordes SP, Blencowe BJ. Essential roles for the splicing regulator nSR100/SRRM4 during nervous system development. Genes & Development. 2015; 29: 746–759.

[33] Zhang X, Coleman IM, Brown LG, True LD, Kollath L, Lucas JM, *et al.* SRRM4 expression and the loss of REST activity may promote the emergence of the neuroendocrine phenotype in castration-resistant prostate cancer. Clinical Cancer Research. 2015; 21: 4698–4708.

[34] Calcinotto A, Spataro C, Zagato E, Di Mitri D, Gil V, Crespo M, *et al.* IL-23 secreted by myeloid cells drives castration-resistant prostate cancer. Nature. 2018; 559: 363–369.

[35] Siegall CB, Schwab G, Nordan RP, FitzGerald DJ, Pastan I. Expression of the interleukin 6 receptor and interleukin 6 in prostate carcinoma cells. Cancer Research. 1990; 50: 7786–7788.

[36] Wang Q, Horiatis D, Pinski J. Interleukin-6 inhibits the growth of prostate cancer xenografts in mice by the process of neuroendocrine differentiation. International Journal of Cancer. 2004; 111: 508–513.

[37] Wang C, Peng G, Huang H, Liu F, Kong D, Dong K, *et al.* Blocking the feedback loop between neuroendocrine differentiation and macrophages improves the therapeutic effects of enzalutamide (MDV3100) on prostate cancer. Clinical Cancer Research. 2018; 24: 708–723.

[38] Ge D, Gao AC, Zhang Q, Liu S, Xue Y, You Z. LNCaP prostate cancer cells with autocrine interleukin-6 expression are resistant to IL-6-induced neuroendocrine differentiation due to increased expression of suppressors of cytokine signaling. Prostate. 2012; 72: 1306–1316.