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EDITORIAL

Gangliocytic paraganglioma: An overview and future perspective

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Abstract

Gangliocytic paraganglioma (GP) is rare neuroendocrine tumor (NET) with a good prognosis that commonly arising from duodenum. Although the tumor is characterized by its unique triphasic cells (epithelioid, spindle, and ganglion-like cells), the proportions of these three tumor cells vary widely from case to case, and occasionally, morphological and immunohistochemical similarities are found between GP and NET G1 (carcinoid tumors). Further, GP accounts for a substantial number of duodenal NETs. Therefore, GP continues to be misdiagnosed, most often as NET G1. However, GP has a better prognosis than NET G1, and it is important to differentiate GP from NET G1. In this article, I wish to provide up-to-date clinicopathological information to help oncologists gain better insight into the diagnosis and clinical management of this tumor.

Key words: Neuroendocrine tumor; Gangliocytic paraganglioma; Progesterone receptor; Pancreatic polypeptide; Literature survey

Core tip: Although gangliocytic paraganglioma (GP) has been regarded as a rare neuroendocrine tumor (NET), GP accounts for a significant number of duodenal NETs. Morphological and immunohistochemical similarities between GP and NET G1 often lead to misdiagnoses of both. However, the prognosis is often better for patients with GP than for those with NET G1. Therefore, it is important to differentiate GP from NET G1. This editorial provides up-to-date data on the clinicopathological characteristics of GP and emphasizes the importance of confirming progesterone receptor and pancreatic polypeptide immunoreactivity for differentiating GP from NET G1.

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INTRODUCTION

Gangliocytic paraganglioma (GP) is rare tumor with a good prognosis that commonly arising from the small intestine (especially, duodenum). Gastrointestinal neuroendocrine tumors (NETs) have a low, but gradually increasing, incidence worldwide[1]. Specifically, the overall prognosis for patients with gastrointestinal NETs has improved and has been favorable[2], but some investigators have reported 5-year survival rates of patients with NET G1 of approximately 80%[3]. Although few patients with liver metastases[4-7] and one with fatal GP[6] have been reported, GP shows a benign course more frequently than NET G1.

Thus, it is important to distinguish between GP and NET G1. However, morphological and immunohistochemical similarities between GP and NET G1 may lead to misdiagnosis[8,9]. Thus, oncologists, clinicians, and pathologists should be aware of the concept of GP because our previous study suggests that GP accounts for a consistent proportion of NETs arising from the duodenum[10]. In this editorial, I would like to discuss the overview and future perspectives of GP, on the basis of our up-to-date systematic review.

Data from 263 patients with GP were collected and analyzed[11]. The vast majority of GPs arose in the duodenum (89.7%). The mean age of patients with GP was 53.5 years. A slight male-to-female predominance was observed, with a ratio of approximately 3:2. Gastrointestinal bleeding and abdominal pain were commonly reported (47.9% and 44.7%, respectively), and many patients were asymptomatic. The mean tumor size was 25.7 mm, and notably, the proportion of the three characteristic GP cells (epithelioid, spindle, and ganglion-like cells) varied considerably from case to case. For a correct diagnosis of GP, pathologists should be aware of the histopathological heterogeneity of this tumor.

Lymph node and liver metastases were observed in approximately 10% and 1% of patients with GP, respectively. Notably, our statistical analysis showed that the depth of invasion was the most significant risk factor for lymph node metastases (tumor size has little effect on lymph node metastasis)[11]. These findings and the associated histological heterogeneity indicate that GP may have hamartomatous characteristics.

To date, pancreaticoduodenectomy is the generally preferred treatment for GP. However, since GP grows slower than NET G1, less invasive procedures (especially endoscopic procedures) have gradually increased in popularity[12]. In fact, in our systematic review, 27 patients underwent endoscopic procedures and showed favorable outcomes, with the exception of one patient who required additional surgery because of a positive surgical margin.

However, to perform less invasive procedures, a definite diagnosis of GP before surgery is essential. Unfortunately, it is difficult to diagnose GP based on a usual biopsy because of the inaccessibility of the tumor (GP is often in a submucosal layer or deeper) and the similarities between GP and NET G1. To solve the first problem, a boring biopsy may be effective because it obtains submucosal tissue. In fact, some patients were successfully diagnosed with GP following multiple boring biopsies[13]. To solve the second problem, I wish to emphasize the usefulness of immunohistochemical examination of pancreatic polypeptide and progesterone receptor levels. GP epithelioid cells show positivity for both markers, and NET G1 shows negativity, and this difference helps distinguish between GP and NET G1. The main differences between GP and NET G1 are summarized in Table 1.

CONCLUSION

Occasionally, GP is misdiagnosed as NET G1, and immunohistochemical examinations of progesterone receptor and pancreatic polypeptide levels help differentiate GPs. Accurate GP identification will facilitate the use of less invasive treatment procedures.

Table 1 Differences in gangliocytic paraganglioma and gastrointestinal neuroendocrine tumor G1

	Gangliocytic paraganglioma	Gastrointestinal neuroendocrine tumor G1
Predominant site of the primary tumor	Duodenum (approximately 90%)	Small intestine, but duodenal is relatively rare
5-yr survival rates	Excellent (approximately 100%)	Good (approximately 80%)
Incidence	Extremely rare	Relatively rare, but gradually increasing, incidence worldwide
Morphological findings obtained by surgery	Epithelioid, spindle, and ganglion-like cells	Nesting, trabecular pattern, and/or rosette formation with nuclear palisading
Immunohistochemistry (pancreatic polypeptide and progesterone receptor)	Epithelioid cells show positive reactivity for both.	Tumor cells show negative reactivity for both
Perspective	Accurate diagnosis of gangliocytic paraganglioma will facilitate the use of less invasive treatment procedures	

REFERENCES

1 **Okubo Y**, Kasajima R, Suzuki M, Miyagi Y, Motohashi O, Shiozawa M, Yoshioka E, Washimi K, Kawachi K, Kameda Y, Yokose T. Risk factors associated with the progression and metastases of hindgut neuroendocrine tumors: a retrospective study. *BMC Cancer* 2017; **17**: 769 [PMID: 29145818 DOI: 10.1186/s12885-017-3769-4]

2 **Shen H**, Yu Z, Zhao J, Li XZ, Pan WS. Early diagnosis and treatment of gastrointestinal neuroendocrine tumors. *Oncol Lett* 2016; **12**: 3385-3392 [PMID: 27900009 DOI: 10.3892/ol.2016.5062]

3 **Pape UF**, Jann H, Müller-Nordhorn J, Bockelbrink A, Berndt U, Willich SN, Koch M, Röcken C, Rindi G, Wiedenmann B. Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors. *Cancer* 2008; **113**: 256-265 [PMID: 18506737 DOI: 10.1002/cncr.23549]

4 **Amin SM**, Albrechtsen NW, Forster J, Damjanov I. Gangliocytic paraganglioma of duodenum metastatic to lymph nodes and liver and extending into the retropancreatic space. *Pathologica* 2013; **105**: 90-93 [PMID: 24047035]

5 **Rowsell C**, Coburn N, Chetty R. Gangliocytic paraganglioma: a rare case with metastases of all 3 elements to liver and lymph nodes. *Ann Diagn Pathol* 2011; **15**: 467-471 [PMID: 21036639 DOI: 10.1016/j.anndiagpath.2010.07.009]

6 **Li B**, Li Y, Tian XY, Luo BN, Li Z. Malignant gangliocytic paraganglioma of the duodenum with distant metastases and a lethal course. *World J Gastroenterol* 2014; **20**: 15454-15461 [PMID: 25386095 DOI: 10.3748/wjg.v20.i41.15454]

7 **Okubo Y**, Yokose T, Motohashi O, Miyagi Y, Yoshioka E, Suzuki M, Washimi K, Kawachi K, Nito M, Nemoto T, Shibuya K, Kameda Y. Duodenal Rare Neuroendocrine Tumor: Clinicopathological Characteristics of Patients with Gangliocytic Paraganglioma. *Gastroenterol Res Pract* 2016; **2016**: 5257312 [PMID: 28096810 DOI: 10.1155/2016/5257312]

8 **Okubo Y**. Gangliocytic Paraganglioma Is Often Misdiagnosed as Neuroendocrine Tumor G1. *Arch Pathol Lab Med* 2017; **141**: 1309 [PMID: 28968157 DOI: 10.5858/arpa.2017-0102-LE]

9 **Okubo Y**. Gangliocytic Paraganglioma: a Diagnostic Pitfall of Rare Neuroendocrine Tumor. *Endocr Pathol* 2017; **28**: 186 [PMID: 28378267 DOI: 10.1007/s12022-017-9481-8]

10 **Okubo Y**, Nemoto T, Wakayama M, Tochigi N, Shinozaki M, Ishiwatari T, Aki K, Tsuchiya M, Aoyama H, Katsura K, Fujii T, Nishigami T, Yokose T, Ohkura Y, Shibuya K. Gangliocytic paraganglioma: a multi-institutional retrospective study in Japan. *BMC Cancer* 2015; **15**: 269 [PMID: 25886293 DOI: 10.1186/s12885-015-1308-8]

11 **Okubo Y**, Yoshioka E, Suzuki M, Washimi K, Kawachi K, Kameda Y, Yokose T. Diagnosis, Pathological Findings, and Clinical Management of Gangliocytic Paraganglioma: A Systematic Review. *Front Oncol* 2018; **8**: 291 [PMID: 30101131 DOI: 10.3389/fonc.2018.00291]

12 **Papaconstantinou D**, Machairas N, Damaskou V, Zavras N, Kontopoulou C, Machairas A. Duodenal gangliocytic paraganglioma, successfully treated by local surgical excision-a case report. *Int J Surg Case Rep* 2017; **32**: 5-8 [PMID: 28213067 DOI: 10.1016/j.ijscr.2017.01.046]

13 **Matsubayashi H**, Ishiwatari H, Matsui T, Fujie S, Uesaka K, Sugiura T, Okamura Y, Yamamoto Y, Ashida R, Ito T, Sasaki K, Ono H. Gangliocytic Paraganglioma of the Minor Papilla of the Duodenum. *Intern Med* 2017; **56**: 1029-1035 [PMID: 28458307 DOI: 10.2169/internalmedicine.56.7812]

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EDITORIAL

Novel mechanism of drug resistance to proteasome inhibitors in multiple myeloma

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Abstract

Multiple myeloma (MM) is a cancer caused by uncontrolled proliferation of antibody-secreting plasma cells in bone marrow, which represents the second most common hematological malignancy. MM is a highly heterogeneous disease and can be classified into a spectrum of subgroups based on their molecular and cytogenetic abnormalities. In the past decade, novel therapies, especially, the first-in-class proteasome inhibitor bortezomib, have been revolutionary for the treatment of MM patients. Despite these remarkable achievements, myeloma remains incurable with a high frequency of patients suffering from a relapse, due to drug resistance. Mutation in the proteasome β5-subunit (PSMB5) was found in a bortezomib-resistant cell line generated *via* long-term coculture with increasing concentrations of bortezomib in 2008, but their actual implication in drug resistance in the clinic has not been reported until recently. A recent study discovered four resistance-inducing PSMB5 mutations from a relapsed MM patient receiving prolonged bortezomib treatment. Analysis of the dynamic clonal evolution revealed that two subclones existed at the onset of disease, while the other two subclones were induced. Protein structural modeling and functional assays demonstrated that all four mutations impaired the binding of bortezomib to the 20S proteasome, conferring different degrees of resistance. The authors further demonstrated two potential approaches to overcome drug resistance by using combination therapy for targeting proteolysis machinery independent of the 20S proteasome.

Key words: Multiple myeloma; Proteasome inhibitor; Bortezomib; Proteasome β5-subunit; Drug resistance; Clonal evolution; Combination therapy

Core tip: Multiple myeloma (MM) is the second common hematological malignancy. An array of new treatments has been approved over the last decade. Hence, the survival of MM patients has improved steadily. Among these new drugs, the first-in-class proteasome inhibitor bortezomib has been revolutionary for targeted therapy. Now bortezomib is the backbone for treating MM. However, emerging drug resistance poses a major challenge for clinicians to use proteasome inhibitors. In this editorial, we discuss proteasome β5-subunit mutations as a novel resistant mechanism to bortezomib and its implication in tracking clonal evolution and suggest potential strategies to overcome drug resistance.

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INTRODUCTION

Multiple myeloma (MM) is the second most common hematological malignancy with approximate 138000 cases worldwide in 2016[1], so it is a significant social and economic burden globally. MM is characterized by uncontrolled proliferation of clonal antibody-secreting plasma cells in bone marrow. The aberrant accumulation of monoclonal proteins in blood and urine causes organ damage, summarized as calcium elevation, renal complications, anemia, and bone lesions[2]. MM is a highly heterogeneous disease and can be classified into a spectrum of subgroups based on their molecular and cytogenetic abnormalities[3]. According to primary genetic events, MM can be divided into hyperdiploid (HD) and non-hyperdiploid (NHD). There are several subgroups within NHD, such as t(11;14)(q13;q32) and CCND3, t(4;14)(p16;q32) and MMSET, FGFR3, t(14;16)(q32;q23), and other MAF translocations. These molecular cytogenetic subgroups and their associated prognosis are summarized in Table 1.

This heterogeneity is one of the factors contributing to the limited effect of chemotherapy (melphalan, cyclophosphamide, and doxorubicin) in general. In the last 12 years, significant progress has been made in the novel therapies for the treatment of MM patients[10]. These new agents, including proteasome inhibitors, immunomodulatory drugs (IMiDs), histone deacetylase (HDAC) inhibitors, and monoclonal antibodies, have significantly improved the survival of standard risk MM patients[11]. Especially, the first-in-class proteasome inhibitor bortezomib has been revolutionary for targeted therapy for MM[12]. Despite remarkable achievements in the past decade, myeloma remains incurable with a high frequency of patients suffering from a relapse, due to primary (inherent) and secondary (acquired) drug resistance[13]. Diversified molecular mechanisms underlying the resistance to proteasome inhibitor have been unveiled, including overexpression of a superfamily of ATP-binding cassette transporters (MDR, MRP1, *etc*.), enhanced aggresomal protein pathway, overexpression of heat shock proteins, bone marrow microenvironment, and appearance of mutations in proteasome subunits[14]. Although mutation in the proteasome β5-subunit (PSMB5) was found in a bortezomib-resistant cell line generated *via* long-term coculture with increasing concentrations of bortezomib in 2008[15], PSMB5 mutation has never been identified in relapsed or refractory MM patients until recently.

STUDY ANALYSIS

In a recent study by Barrio *et al*[16], four PSMB5 mutations from a MM patient receiving prolonged bortezomib treatment have been discovered and functionally validated. These investigators performed targeted deep-sequencing of 88 MM-related genes (M^3P panel) on paired tumor-germline samples from 161 multi-refractory MM patients. They reported four subclonal mutations in PSMB5 gene: c.235G>A (p.A20T), c.256G>C (p.A27P), c.312G>C (p.M45I), and c.365G>A (p.C63Y) (protein positions

Table 1 Molecular cytogenetic classification of multiple myeloma and prognosis

Chromosomes affected (gene)	Ploidy	Prognosis	Ref.
t (11;14) (*CCND3*)	NH	Good	[4]
t (14;16) (*c-MAF*)	NH	Poor	[5]
t (4;14) (*FGFR3* and *NSD2*)	NH/H	Poor	[6,7]
Other IgH	NH/H	Poor	[8]
Hyperdiploidy	H	Good	[9]

IgH: Immunoglobulin heavy chain; H: Hyperdiploidy; NH: Non-hyperdiploidy.

refer to the cleaved mature protein). These mutations were further confirmed by whole exome sequencing. Interestingly, these subclonal lines were still sensitive to the combination of pomalidomide and elotuzumab as analysis of clonal evolution at different time points (TP) revealed that two subclonal lines (C63Y and A27P) become undetectable at TP4 and the remaining M45I and A20T also disappeared at TP5 (5 months later than TP4). Tracing back the samples available at TP1 (diagnosis) and TP2 (first relapse) confirmed the pre-existence of two of the variants, c.235G>A and c.365G>A, at these two earlier TPs. The illustration of the temporal order of clonal evolutionary trajectory in this patient adds to our growing understanding of MM evolution and therapeutic resistance. The co-existence of emergent new subclones after selection pressure (bortezomib treatment) on the original subclones confirms the "Big Bang" model of cancer evolution in a branching rather than in a "step-wise" linear progression[17,18]. Furthermore, the eradication of all these four subclones after a combination regime including the second-generation IMiD pomalidomide and the immunostimulatory monoclonal antibody elotuzumab underpins the importance of developing novel drugs for relapsed MM patients.

Notably, all four mutations occurred within a highly conserved region in exon 2, and protein structural analysis demonstrated that the mutations are located either within the S1 pocket (A20T, A27P, and M45I) or in proximity to the substrate-binding channel (C63Y). The authors performed *in vitro* functional assays and all the mutants impaired the binding of bortezomib to the proteasome, reduced catalytic proteasome activity, and conferred resistance to bortezomib and other proteasome inhibitors to varying degrees. Importantly, these PSMB5 mutant lines remain sensitive to p97/VCP AAA ATPase inhibitor, CB5083, which blocks proteolysis machinery independent of 20S proteasome. These results highlight another approach to overcome drug resistance to proteasome inhibitors by using p97/VCP inhibitors.

In conclusion, this study not only validated the importance of PSMB5 in mediating drug resistance to proteasome inhibitors, but also deciphered the dynamic and temporal effect of clonal evolution in the development of resistance and deepened our understanding of the relationship between clonal evolution and drug resistance in MM cells.

CONCLUSION

Drug resistance has been implicated in 90% of MM-related deaths, which poses a daunting challenge in the management of MM. The combination of the second-generation IMiD and antibody therapy or novel agents targeting proteasome-independent proteolysis machinery can override the resistance to proteasome inhibitors. These approaches hold promise to further improve the survival of relapsed and refractory MM patients. However, we have to wait for well-designed clinical trials to validate its efficacy and evaluate the toxicity. In addition, prospective biomarkers for prediction of drug resistance are absent. Owing to the heterogeneity of MM and various mechanisms involved in resistance, it is unlikely that one biomarker fits all MM. Nevertheless, screening PSMB5 mutations at diagnosis, during the treatment, and subsequent follow-ups should be useful in monitoring drug resistance to proteasome inhibitors. Furthermore, some important questions remain unanswered, for example, whether mutations in other 20S proteasome subunits, like PSMA5, exist. Finally, single-cell sequencing technology is particularly useful in tracking clonal evolution, providing opportunities to characterize MM subclones in unprecedented detail. We now have a better chance to conquer drug resistance and significantly further improve the outcome of MM patients.

REFERENCES

1 **Cowan AJ**, Allen C, Barac A, Basaleem H, Bensenor I, Curado MP, Foreman K, Gupta R, Harvey J, Hosgood HD, Jakovljevic M, Khader Y, Linn S, Lad D, Mantovani L, Nong VM, Mokdad A, Naghavi M, Postma M, Roshandel G, Shackelford K, Sisay M, Nguyen CT, Tran TT, Xuan BT, Ukwaja KN, Vollset SE, Weiderpass E, Libby EN, Fitzmaurice C. Global Burden of Multiple Myeloma: A Systematic Analysis for the Global Burden of Disease Study 2016. *JAMA Oncol* 2018; **4**: 1221-1227 [PMID: 29800065 DOI: 10.1001/jamaoncol.2018.2128]

2 **Kyle RA**, Rajkumar SV. Multiple myeloma. *Blood* 2008; **111**: 2962-2972 [PMID: 18332230 DOI: 10.1182/blood-2007-10-078022]

3 **Chng WJ**, Glebov O, Bergsagel PL, Kuehl WM. Genetic events in the pathogenesis of multiple myeloma. *Best Pract Res Clin Haematol* 2007; **20**: 571-596 [PMID: 18070707 DOI: 10.1016/j.beha.2007.08.004]

4 **Fonseca R**, Blood EA, Oken MM, Kyle RA, Dewald GW, Bailey RJ, Van Wier SA, Henderson KJ, Hoyer JD, Harrington D, Kay NE, Van Ness B, Greipp PR. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood* 2002; **99**: 3735-3741 [PMID: 11986230 DOI: 10.1182/blood.V99.10.3735]

5 **Narita T**, Inagaki A, Kobayashi T, Kuroda Y, Fukushima T, Nezu M, Fuchida S, Sakai H, Sekiguchi N, Sugiura I, Maeda Y, Takamatsu H, Tsukamoto N, Maruyama D, Kubota Y, Kojima M, Sunami K, Ono T, Ri M, Tobinai K, Iida S. t(14;16)-positive multiple myeloma shows negativity for CD56 expression and unfavorable outcome even in the era of novel drugs. *Blood Cancer J* 2015; **5**: e285 [PMID: 25723856 DOI: 10.1038/bcj.2015.6]

6 **Chesi M**, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. *Blood* 1998; **92**: 3025-3034 [PMID: 9787135 DOI: 10.1016/S0006-355X(99)80021-3]

7 **Kalff A**, Spencer A. The t(4;14) translocation and FGFR3 overexpression in multiple myeloma: prognostic implications and current clinical strategies. *Blood Cancer J* 2012; **2**: e89 [PMID: 22961061 DOI: 10.1038/bcj.2012.37]

8 **Cleynen A**, Szalat R, Kemal Samur M, Robiou du Pont S, Buisson L, Boyle E, Chretien ML, Anderson K, Minvielle S, Moreau P, Attal M, Parmigiani G, Corre J, Munshi N, Avet-Loiseau H. Expressed fusion gene landscape and its impact in multiple myeloma. *Nat Commun* 2017; **8**: 1893 [PMID: 29196615 DOI: 10.1038/s41467-017-00638-w]

9 **Chretien ML**, Corre J, Lauwers-Cances V, Magrangeas F, Cleynen A, Yon E, Hulin C, Leleu X, Orsini-Piocelle F, Blade JS, Sohn C, Karlin L, Delbrel X, Hebraud B, Roussel M, Marit G, Garderet L, Mohty M, Rodon P, Voillat L, Royer B, Jaccard A, Belhadj K, Fontan J, Caillot D, Stoppa AM, Attal M, Facon T, Moreau P, Minvielle S, Avet-Loiseau H. Understanding the role of hyperdiploidy in myeloma prognosis: which trisomies really matter? *Blood* 2015; **126**: 2713-2719 [PMID: 26516228 DOI: 10.1182/blood-2015-06-650242]

10 **Ria R**, Reale A, Vacca A. Novel agents and new therapeutic approaches for treatment of multiple myeloma. *World J Methodol* 2014; **4**: 73-90 [PMID: 25332907 DOI: 10.5662/wjm.v4.i2.73]

11 **Larocca A**, Mina R, Gay F, Bringhen S, Boccadoro M. Emerging drugs and combinations to treat multiple myeloma. *Oncotarget* 2017; **8**: 60656-60672 [PMID: 28948001 DOI: 10.18632/oncotarget.19269]

12 **Grosicki S**, Barchnicka A, Jurczyszyn A, Grosicka A. Bortezomib for the treatment of multiple myeloma. *Expert Rev Hematol* 2014; **7**: 173-185 [PMID: 24617331 DOI: 10.1586/17474086.2014.899144]

13 **Robak P**, Drozdz I, Szemraj J, Robak T. Drug resistance in multiple myeloma. *Cancer Treat Rev* 2018; **70**: 199-208 [PMID: 30245231 DOI: 10.1016/j.ctrv.2018.09.001]

14 **Manasanch EE**, Orlowski RZ. Proteasome inhibitors in cancer therapy. *Nat Rev Clin Oncol* 2017; **14**: 417-433 [PMID: 28117417 DOI: 10.1038/nrclinonc.2016.206]

15 **Oerlemans R**, Franke NE, Assaraf YG, Cloos J, van Zantwijk I, Berkers CR, Scheffer GL, Debipersad K, Vojtekova K, Lemos C, van der Heijden JW, Ylstra B, Peters GJ, Kaspers GL, Dijkmans BA, Scheper RJ, Jansen G. Molecular basis of bortezomib resistance: proteasome subunit beta5 (PSMB5) gene mutation and overexpression of PSMB5 protein. *Blood* 2008; **112**: 2489-2499 [PMID: 18565852 DOI: 10.1182/blood-2007-08-104950]

16 **Barrio S**, Stühmer T, Da-Viá M, Barrio-Garcia C, Lehners N, Besse A, Cuenca I, Garitano-Trojaola A, Fink S, Leich E, Chatterjee M, Driessen C, Martinez-Lopez J, Rosenwald A, Beckmann R, Bargou RC, Braggio E, Stewart AK, Raab MS, Einsele H, Kortüm KM. Spectrum and functional validation of PSMB5 mutations in multiple myeloma. *Leukemia* 2019; **33**: 447-456 [PMID: 30026573 DOI: 10.1038/s41375-018-0216-8]

17 **Sottoriva A**, Kang H, Ma Z, Graham TA, Salomon MP, Zhao J, Marjoram P, Siegmund K, Press MF, Shibata D, Curtis C. A Big Bang model of human colorectal tumor growth. *Nat Genet* 2015; **47**: 209-216 [PMID: 25665006 DOI: 10.1038/ng.3214]

18 **Keats JJ**, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, Van Wier S, Blackburn PR, Baker AS, Dispenzieri A, Kumar S, Rajkumar SV, Carpten JD, Barrett M, Fonseca R, Stewart AK, Bergsagel PL. Clonal competition with alternating dominance in multiple myeloma. *Blood* 2012; **120**: 1067-1076 [PMID: 22498740 DOI: 10.1182/blood-2012-01-405985]

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ORIGINAL ARTICLE

Observational Study

DNA extraction from paraffin embedded colorectal carcinoma samples: A comparison study of manual *vs* automated methods, using four commercially kits

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Abstract

BACKGROUND

Nucleic acid isolation from formalin-fixed, paraffin-embedded tissue (FFPET) samples is a daily routine in molecular pathology laboratories, but extraction from FFPET is not always easily achieved. Choosing the right extraction technique is key for further examinations.

AIM

To compare the performance of four commercially available kits used for DNA extraction in routine practice.

METHODS

DNA isolation was performed on 46 randomly selected formalin-fixed, paraffin-embedded (FFPE) colorectal adenocarcinoma (CRC) surgical specimens. Four commercially available extraction kits were used: two for manual DNA extraction (the PureLink Genomic DNA Mini Kit from Invitrogen and the High Pure FFPE DNA Isolation Kit from Roche) and two for automated DNA extraction (the iPrep Genomic DNA Kit from Invitrogen and the MagnaPure LC DNA Isolation Kit from Roche). The DNA concentration and quality (odds ratio) among the four systems were compared. The results were correlated with the clinicopathological aspects of CRC cases: age, gender, localization, macro- and microscopic features, lymph node metastases, and the lymph node ratio.

RESULTS

The highest DNA concentration was obtained using the manual kits: 157.24 ±

authors have declared no conflicts of interest.

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62.99 ng/μL for the PureLink Genomic DNA Mini Kit and 86.64 ng/μL ± 43.84 for the High Pure FFPE DNA Isolation Kit ($P < 0.0001$). Lower concentrations were obtained with automated systems: 20.39 ± 21.19 ng/μL for the MagnaPure LC DNA Isolation Kit and 8.722 ± 6.408 ng/μL for the iPrep Genomic DNA Kit, with differences between the systems used ($P < 0.0001$). The comparison between age, gender, tumor localization, pT or pN stage and the lymph node ratio indicated no statistically significant difference in DNA concentration using any of the nucleic acid isolation kits. DNA concentration was influenced by the macroscopic features and grade of differentiation. A higher DNA concentration was obtained for well-differentiated polypoid colorectal adenocarcinomas (CRCs), compared with undifferentiated ulcero-infiltrative carcinomas, irrespective of the kit used.

CONCLUSION

For research or diagnosis that needs high DNA concentrations, manual methods of DNA isolation should be used. A higher amount of DNA can be obtained from polypoid-type differentiated CRCs. Automated systems confer comfort and a lower amount of DNA that is, however, sufficient for classic polymerase chain reaction (PCR) and real-time quantitative PCR molecular examinations. All four commercially available kits can be successfully used in daily practice.

Key words: DNA isolation; Colorectal cancer; Paraffin-embedded; PureLink Genomic DNA Mini Kit; High Pure FFPE DNA Isolation Kit; iPrep Genomic DNA Kit; MagnaPure LC DNA Isolation Kit

Core tip: In this paper we proved the advantages and disadvantages of manual *vs* automated methods of DNA extraction. The original aspect refers to the correlation between DNA concentration and colorectal adenocarcinoma (CRC) features. We conclude that manual methods are more suitable for obtaining high DNA concentrations, especially from differentiated polypoid-type CRCs. In CRC samples, a higher DNA concentration is associated with a lower OD value.

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INTRODUCTION

Friedrich Miescher[1] performed the first isolation of DNA in 1868/1869. In 1988, Miller *et al*[2] described the simple salting-out procedure of DNA extraction from human cells compared to the classic phenol-chloroform method. They found that the salting-out procedure was as good as the classic method using chloroform. In 1991, Lahiri *et al*[3] demonstrated that the salting-out procedure is even better for RFLP (restriction fragment length polymorphism). Regarding the type of preserved tissue, although fresh tissues are preferred, Goelz *et al*[4] demonstrated in 1985 that DNA can also be isolated from formalin-fixed, paraffin-embedded tissues (FFPETs).

Irrespective of the source of commercially available kits, manual nucleic acid isolation methods are based on the same principle: cells must be disrupted and digested with Proteinase K and proteins and other contaminants need to be washed out in order to achieve pure DNA. Extraction from FFPETs needs an additional deparaffinization step with 100% xylene in order to get rid of the paraffin[5].

Automated magnetic bead methods are time-saving procedures. Starting from sample lysis to DNA elution, everything is done by a machine. However, isolation from formalin-fixed, paraffin-embedded tissue requires the same additional step as manual methods, namely deparaffinization[6].

The aim of this paper was to compare the advantages and disadvantages of four DNA extraction kits used in daily practice for DNA isolation from formalin-fixed, paraffin-embedded (FFPE) colorectal adenocarcinoma (CRC) surgical specimens: two manual and two automated magnetic bead kits. An analysis of the correlation between the results and the clinicopathological features of colorectal adenocarcinomas (CRCs) was also conducted.

MATERIALS AND METHODS

Forty-six consecutive cases of CRC were randomly selected for DNA isolation, with the approval of the Ethical Committee of Clinical County Emergency Hospital and the University of Medicine, Pharmacy, Science and Technology of Tirgu-Mures, Romania. No preoperative chemo- or radiotherapy was administered in any of the examined cases. The used paraffin blocks from CRC surgical specimens were archived at the Department of Pathology of Clinical County Emergency Hospital of Tirgu-Mures, Romania, during the period 2010-2015.

Tissue preparation

DNA was extracted from FFPE-CRCs. Hematoxilin and eosin stains were first performed to mark the most appropriate area. The selection of the tumor area was based on the presence of tumor cells in over 80% of the marked tissue, without necroses, hemorrhages, inflammatory or highly fibrotic stroma. After macrodissection of the tumor, 3 x 5 μm sections were created and inserted into 1.5 mL Eppendorf SafeLock tubes (Eppendorf, Hamburg, Germany).

DNA isolation with manual systems

For manual DNA isolation, two commercially available kits were used: the PureLink Genomic DNA Mini Kit from Invitrogen Carlsbad, CA 92008, United States and the High Pure FFPE DNA Isolation Kit from Roche GmbH, Mannheim, Germany. For each of the 46 included cases, two manual isolations per case were performed according to the manufacturers' protocols (Table 1).

Invitrogen kits were tested at the Pathology Department of Mures County Hospital, while Roche kits were tested at the Molecular Pathology Laboratory of the National Institute of Oncology, Budapest. The same team performed all isolations.

The manual methods (for both kits) were performed on an anion-exchange resin. It is a macroporous silica-based resin with a high density of diethylaminoethyl (DEAE) groups. The purification is based on the interaction between the negatively charged phosphates of the nucleic acid backbone and the positively charged DEAE groups on the surface of the resin[7].

DNA isolation with automated methods

For automated DNA isolation, two commercially available automated magnetic bead kits were used: the iPrep Genomic DNA kit from Invitrogen and the MagnaPure LC DNA Isolation kit from Roche. Similar to the manual methods, for each of the 46 included cases, two automated isolations per case were performed according to the manufacturers' protocols (Table 1).

Both of the automated purification techniques use magnetic bead isolation principles. Positively charged magnetic beads can form an ionic bond with the negatively charged DNA backbone at low pH values. At high pH values, the magnetic beads lose their charge and DNA binding ability. In deparaffinized tissues, after a standard automatic tissue lysis step which takes 15 min, the genomic DNA is isolated in a 15-minute procedure that involves binding the genomic DNA to the magnetic beads in a low pH buffer, immobilizing the beads with a magnet, washing and finally, elution in a higher pH buffer (Table 1).

DNA concentration and quality

DNA parameters (concentration and quality) were determined using a Nanodrop machine (ThermoScientific, United States). Readings were taken at wavelengths of 260 nm and 280 nm. The optical density (OD) ratio (A260/A280) was automatically calculated.

As a standard parameter for purity, an OD ratio value of 1.8-2.0 was used. A ratio less than 1.8 indicated protein contamination, while a ratio above 2.0 indicated contamination by chloroform, phenol or other organic compounds.

Statistical analysis

Statistical analysis of the data took into account the DNA parameters, which were compared for all four systems used. They were then correlated with tumor

Table 1 DNA isolation protocols

PureLink Genomic DNA mini kit (Invitrogen–ma-nual)	Time	iPrep genomic DNA kit (Invitrogen–aut-omated)	Time	High Pure FFPE DNA isolation kit (Rochemanual)	Time	MagnaPure LC DNA Isolation kit (Roche–automated)	Time
3 sections of 5 µm	5-10 min	3 sections of 5 µm	5-10 min	3 sections of 5 µm	5-10 min	3 sections of 5 µm	5-10 min
Deparaffinizatio-n using Xylol 3 times	3 x 10 min	Deparaffinization using Xylol 3 times	3 x 10 min	Deparaffinization using Xylol 3 times	3 x 10 min	Deparaffinization using Xylol 3 times	3 x 10 min
Lysis and digestion (20 µL of ProtK)	From 30 min to overnight	Sample lysis		Lysis and digestion (70 µL ProtK)	30-90 min	Sample lysis and binding	
Binding DNA to silica membranes (columns)	15 min	Binding DNA to magnetic beads	30 min	Binding DNA to silica membranes (columns)	15 min	ProtK	30 min
Washing out contaminants	15 min	Magnetic separation of beads		Washing out contaminants	15 min	Binding DNA to beads	
Eluting	3 min	Separation of liquid solutions from beads Washing beads eluting		Eluting	3 min	Washing out contaminants Washing beads eluting	
~ 1.96 Euro/sample		~ 7.8 Euro/sample		~ 3.66 Euro/sample		~ 6.81 Euro/sample	

localization, macroscopic and microscopic features, the depth of infiltration, the lymph node ratio, and the tumor stage, which were determined according to the latest classification rules[8]. Chi-squared and Fisher's exact tests were used for statistical analysis using GraphPad Prism 8.0.1 software. A *P* value lower than 0.05 with a 95% confidence interval (CI) was considered statistically significant. GraphPad Prism 8.0.1 software, using Chi Square test and Fisher's exact test, was used for statistically assessment. A *P* value lower than 0.05, at 95%CI, was considered statistically significant.

RESULTS

Manual systems

In all 46 cases, after the deparaffinization step with xylene, the time required for manual DNA isolation for both manual kits according to the manufacturers' protocols (Table 1) ranged from 60 min to over 12 h when overnight lysis was necessary. This time was respected for the isolation of a few probes (< 5). In cases of incomplete lysis, re-centrifugation was conducted and Proteinase K was added. These supplementary steps prolonged the isolation time irrespective of the kit used.

The major difference between the two manual isolation kits in terms of the indicated protocol (Table 1) is the quantity of Proteinase K that should be added to the tissue lysis buffer. While Invitrogen suggests using 20 µL of Proteinase K, Roche indicates 70 µL.

The average DNA concentration isolated with the Invitrogen manual kit was 157.24 ± 62.99 ng/µL (37.6-316 ng/µL), while with the Roche kit a lower median value was obtained ($P < 0.0001$) at 86.64 ± 43.84 ng/µL (4.2-168.9 ng/µL). In three of the 46 cases, a higher DNA concentration was obtained with the Roche kit, compared with the Invitrogen manual kit (Table 2).

DNA purity was adequate at 1.8-2.0, without any protein or organic compound contamination, irrespective of the method used. Only four out of the 46 cases had an OD ratio lower than 1.8. A significant difference in the OD value was found between the two manual methods ($P = 0.019$).

In three of the 46 probes (6.46%), the OD ratio was lower than 1.8 using the Roche manual kit, while using the Invitrogen kit, 16 of the 46 DNA samples (37.78%) had a low OD value. A higher OD value (> 2.00) was found in 29 of the 46 cases using the

Table 2 DNA concentration (ng/μL) using four commercially kits for DNA isolation

	PureLink Genomic DNA mini kit (Invitrogen–manual)	iPrep genomic DNA kit (Invitrogen–automated)	MagnaPure LC DNA Isolation kit (Roche–automated)	High Pure FFPE DNA isolation kit (Roche–manual)
Mean	157.24	8.72	20.39	86.64
Standard deviation	62.99	6.40	21.19	43.84
Minimum	37.60	0.70	0.30	4.20
Maximum	316	29.80	121	168.90

Roche system and in no cases using the Invitrogen system (Table 3).

Automated methods

For both automated methods, the protocol indicated by Invitrogen and Roche, using the magnetic beads principle, was similar (Table 1). For one run, the total time was 30 min for 12 probes with the iPrep Genomic DNA kit from Invitrogen and 30 min for 11 probes with the MagnaPure LC DNA Isolation Kit from Roche. For each run, one template control was used to check the probes for contamination.

Compared with the manual kits, the DNA concentration obtained was significantly lower ($P < 0.0001$) irrespective of the automatic system used (Figure 1).

A significantly lower ($P < 0.0001$) DNA concentration (8.72 ± 6.4 ng/μL, 0.70-29.80) was obtained with the automatic iPrep Genomic DNA Kit from Invitrogen, compared with the automatic MagnaPure LC DNA Isolation Kit from Roche (20.39 ± 21.19 ng/μL, 0.30-121) (Tables 2 and 3).

Regarding DNA purity, no significant difference in the OD value was found between the two automated methods ($P = 0.56$).

In 19 of the 46 probes (41.30%), the OD ratio was lower than 1.8 using the Roche automated system, while using the Invitrogen automated system, 21 of the 46 DNA samples (45.65%) had low OD values. Higher OD values (> 2) were found in 12 of the 46 cases using the Roche system and in four of the 46 cases using the Invitrogen system (Table 3).

Compared to manual isolation methods, the OD values obtained with automated systems were similar for Invitrogen kits ($P = 0.32$), whereas automated DNA extraction was associated with lower OD values ($P < 0.0001$).

Clinicopathological factors and DNA parameters

The comparison between age, gender, tumor localization, the depth of infiltration (pT), lymph node status (pN stage), and the lymph node ratio found no statistically significant difference in DNA concentration using any of the nucleic acid isolation kits (Tables 4-7).

DNA concentration was influenced by the macroscopic aspects and grade of differentiation. A higher concentration of DNA was obtained for polypoid in comparison to ulcero-infiltrative carcinomas, with both Roche systems (Tables 6 and 7) and using the automated system from Invitrogen (Table 5). The manual kit from Invitrogen allowed good concentrations to be extracted, but in half of the cases (23 of 46 cases) a value below 150 ng/μL was obtained (Table 4). For this reason, the *P* value was considered to be at the limit of statistical significance.

Regarding the microscopic aspect of CRC, the concentration of nucleic acids was higher in well-differentiated (G1) carcinomas, compared with G2 + G3 cases (Tables 4-7).

DISCUSSION

In FFPETs, after deparaffinization, the first step in DNA isolation is cell disruption/lysis[3]. After DNA exposure, membrane lipid removal is conducted by adding detergents, proteins and even proteases (an optional step, but almost always included). Precipitation of the DNA is then performed with alcohol (usually ice-cold ethanol or isopropanol). At the end of the procedure, solubilizing the DNA must be conducted in an alkaline buffer or in ultra-pure water.

During DNA isolation, a chelating agent can be added in order to bind divalent cations and stop DNase activities. Cellular or histone proteins bound to DNA can be removed by adding a protease or by precipitating proteins with sodium/ammonium acetate, or extracting them with a phenol-chloroform mixture prior to the DNA

Table 3 DNA quality[1] using four commercially kits for DNA isolation

	PureLink Genomic DNA mini kit (Invitrogen–manual)	iPrep genomic DNA kit (Invitrogen–automated)	MagnaPure LC DNA Isolation kit (Roche–automated)	High Pure FFPE DNA isolation kit (Roche–manual)
Mean	1.80	1.78	1.84	2.19
Standard deviation	0.04	0.14	0.52	0.37
Minimum	1.67	1.42	0.99	1.60
Maximum	3.10	2.25	4.36	3.10

[1]Quality–OD: A260/A280.

precipitation.

The most commonly used protease in DNA extraction is Proteinase K (protease K or endopeptidase K), which is a broad-spectrum serine protease. It digests and removes proteins as a nucleic acid decontamination step. Proteinase K also inactivates nucleases that might induce DNA or RNA degradation during DNA purification. In this study, it was observed that protein contamination was the same when manual protocols were used, highlighting the fact that it is not affected by the amount of Proteinase K (20 μL *vs* 70 μL). On the other hand, the manual probes showed a higher median DNA concentration (157 ng/μL *vs* 87 ng/μL). Irrespective of the manufacturer, the automated DNA extraction was associated with a higher protein contamination rate (OD < 1.8). In these cases, it related to a shorter Proteinase K exposure time, which cannot be modified in-house. Better tissue lysis might induce a lower protein contamination rate.

One original aspect that could be useful in daily practice concerns the correlation obtained in this study between DNA concentration and the clinicopathological parameters of CRCs. Patient age and gender did not influence the DNA concentration, as well as most of the tumor parameters (localization, macroscopic features, pT and pN stage, and lymph node ratio).

We successfully proved that the highest concentration of DNA can be obtained from FFPE well-differentiated CRCs with a polypoid aspect, irrespective of their localization. As ulcero-infiltrative tumors are usually associated with a higher grade of macroscopic lysis, this parameter can influence DNA parameters.

Tumor dedifferentiation might be associated with a high cell division rate[9,10], which could lead to a lower rate of successful DNA lysis.

There are several commercial kits available that include manual and automated isolation procedures. However, although time-consuming, nucleic acid isolation can be done by in-house preparation of all the buffers and solutions necessary for extraction[9,10]. The method used should take into account the quantity of DNA needed [*e.g.*, for adductomics studies or polymerase chain reaction (PCR)] but also the human component, as manual systems need to be managed by well-prepared technicians or biologists.

Fully automated methods can be used successfully for PCR reactions. Although the DNA concentration obtained is lower than by manual methods, it is sufficient for PCR. The costs are higher than for the manual methods.

All of the probes from this study were successfully amplified for real-time PCR reactions. The literature data show that both DNA and RNA can be isolated by automated methods from FFPETs[11-13]. The authors applied a fully automated xylene-free isolation with iron oxide beads coated with a nanolayer of silica[11-13].

An important step in performing DNA isolation from FFPETs is the pre-isolation protocol. Deparaffinization can be performed in tubes (such as in this study) or using slide-digestion (overnight or 72 h) based on in-house protocols. Both methods can be successfully adopted. DNA concentration obtained after 72 h on slide-deparaffinization can be over 500 ng/μL[11-13].

In 2015, Kocjan *et al*[14] compared 69 commercially available DNA extraction kits from 43 companies. They showed that deparaffinization and supplementary lysis can induce a lower DNA concentration[14]. In this study, we have shown that a lower amount of Proteinase K with longer tissue exposure (which is possible for manual kits) leads to a higher concentration of DNA. Although manual extraction confers a higher yield and DNA concentration, automated isolation will replace it in short time, when the costs decrease significantly[15].

The unresolved issue refers to the imbalance between concentration and quality. We have obtained a reverse correlation between concentration and OD value, which could help researchers in their decisions regarding the most appropriate methods

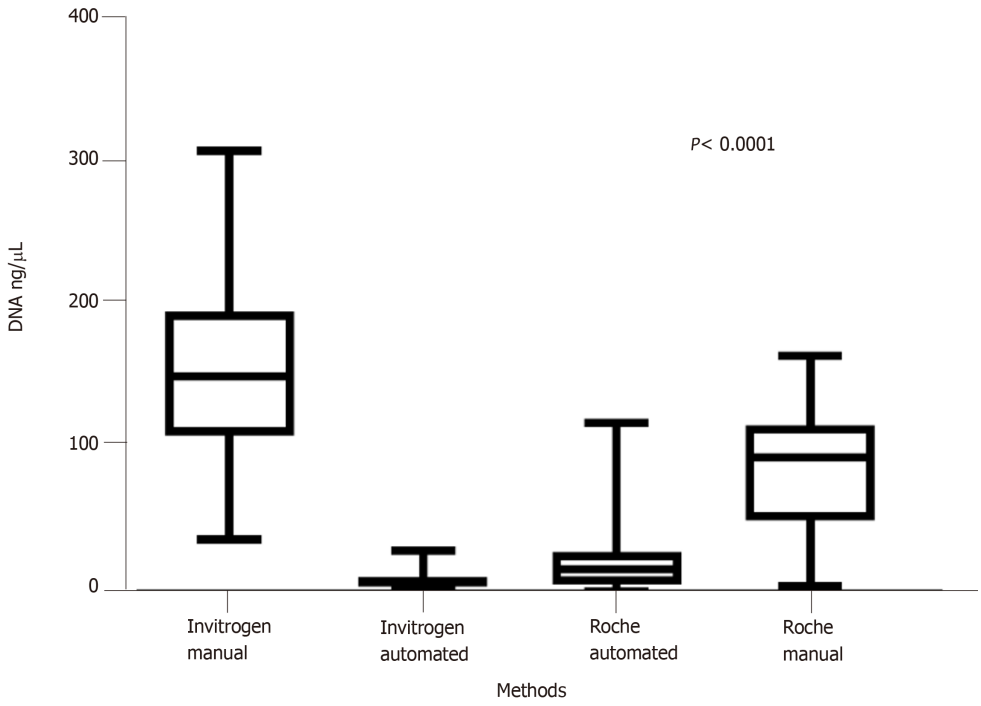

Figure 1 Comparison of four commercially kits show that a higher DNA concentration was obtained with manual, compared with automated methods.

(manual *vs* automatic) and provide explanations for the understandable problems encountered daily in the laboratory. Similar to our findings, it was previously demonstrated that DNA integrity is higher with manual purification, for both tissues and whole blood[16].

The present study has some limitations. Firstly, the number of included cases is small and originates from a single department, with the same techniques used for tissue preparation. Secondly, only CRC samples were used. The above-mentioned aspects should be investigated in larger cohorts with sample size calculations.

In conclusion, the results of this single-center study highlight the importance of the quality of nucleic acid isolation techniques. Manual methods proved to be more controllable and permit in-house adaptation of the protocol, while the obtained DNA concentrations and purity were higher. On the other hand, automated methods are a time-saving option for PCR and real-time quantitative PCR reactions. For CRC samples, it is expected that a higher DNA concentration would be obtained from differentiated polypoid carcinomas.

Table 4 Correlation between DNA concentration[1] and clinicopathological aspects of colorectal cancer

Characteristics		Number	DNA concentration PureLink Genomic DNA mini kit (manual)		*P* value
			≤ 150 ng/μL	> 150 ng/μL	
Age	≤ 60	20	12	8	0.37[3]
	> 60	26	11	15	
Gender	Male	26	15	11	0.37[3]
	Female	20	8	12	
Macroscopic aspect	Polypoid	10	4	15	0.0010[3]
	Ulcero-infiltrative	36	19	8	
Microscopic aspect	G1	13	3	10	0.04[3]
	G2 + G3	33	20	13	
Localization	Proximal colon	8	5	3	0.89[2]
	Distal colon	21	11	10	
	Rectum	17	7	10	
Depth of infiltration (pT stage)	T2-T3 T4	40 6	20 3	20 3	> 0.99[2]
Lymph node metastasis (pN stage)	Present (pN1-3)	17	13	4	0.06[3]
	Absent (pN0)	29	10	19	
Lymph node ratio	< 0.15	36	17	19	0.72[3]
	≥ 0.15	10	6	4	

[1]Manual PureLink Genomic DNA mini kit-Invitrogen.
[2]Chi square test.
[3]Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.

Table 5 Correlation between DNA concentration[1] and clinicopathological aspects of colorectal cancer

Characteristics		Number	iPrep genomic DNA kit (magnetic beads)		*P* value
			≤ 10 ng/μL	> 10 ng/μL	
Age	≤ 60	20	12	8	0.09[3]
	> 60	26	22	4	
Gender	Male	26	19	7	0.99[3]
	Female	20	15	5	
Macroscopic aspect	Polypoid	10	3	7	0.0004[3]
	Ulcero-infiltrative	36	31	5	
Microscopic aspect	G1	13	4	9	< 0.0001[3]
	G2 + G3	33	30	3	
Localization	Proximal colon	8	6	2	0.52[2]
	Distal colon	21	17	4	
	Rectum	17	11	6	
Depth of infiltration (pT stage)	T2-T3 T4	40 6	28 6	12 0	0.11[2]
Lymph node metastasis (pN stage)	Present (pN1-3)	17	14	3	0.31[3]
	Absent (pN0)	29	20	9	
Lymph node ratio	< 0.15	36	25	11	0.25[3]
	≥ 0.15	10	9	1	

[1]Automated iPrep genomic DNA kit-Invitrogen.
[2]Chi square test.
[3]Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.

Table 6 Correlation between DNA concentration[1] and clinicopathological aspects of colorectal cancer

Characteristics		Number	High Pure FFPE DNA isolation kit (manual)		*P* value
			< 100 ng/μL	≥ 100 ng/μL	
Age	≤ 60	20	12	8	0.99[3]
	> 60	26	15	11	
Gender	Male	26	15	11	0.99[3]
	Female	20	12	8	
Macroscopic aspect	Polypoid	10	8	17	0.12[3]
	Ulcero-infiltrative	36	19	2	
Microscopic aspect	G1	13	3	10	0.002[3]
	G2 + G3	33	24	9	
Localization	Proximal colon	8	5	3	0.96[2]
	Distal colon	21	12	9	
	Rectum	17	10	7	
Depth of infiltration (pT stage)	T2-T3 T4	40 6	24 3	16 3	0.64[2]
Lymph node metastasis (pN stage)	Present (pN1-3)	17	8	9	0.35[3]
	Absent (pN0)	29	19	10	
Lymph node ratio	< 0.15	36	23	13	0.27[3]
	≥ 0.15	10	4	6	

[1]Manual High Pure FFPE DNA isolation kit-Roche.
[2]Chi square test.
[3]Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.

Table 7 Correlation between DNA concentration[1] and clinicopathological aspects of colorectal cancer

Characteristics		*P* value	MagnaPure LC DNA Isolation kit (magnetic beads)		*P* value
			< 20 ng/μL	≥ 20 ng/μL	
Age	≤ 60	20	12	8	0.38[3]
	> 60	26	12	14	
Gender	Male	26	12	14	0.38[3]
	Female	20	12	8	
Macroscopic aspect	Polypoid	10	8	2	0.04[3]
	Ulcero-infiltrative	36	16	20	
Microscopic aspect	G1	13	4	9	0.02[3]
	G2 + G3	33	23	10	
Localization	Proximal colon	8	3	5	0.60[2]
	Distal colon	21	11	10	
	Rectum	17	10	7	
Depth of infiltration (pT stage)	T2-T3	40	20	20	0.44[2]
	T4	6	4	2	
Lymph node metastasis (pN stage)	Present (pN1-3)	17	5	12	0.03[3]
	Absent (pN0)	29	19	10	
Lymph node ratio	< 0.15	36	18	18	0.72[3]
	≥ 0.15	10	6	4	

[1]Automated MagnaPure LC DNA Isolation kit-Roche.
[2]Chi square test.
[3]Fisher's exact test. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated.

ARTICLE HIGHLIGHTS

Research background

Nucleic acid isolation from formalin-fixed, paraffin-embedded tissue (FFPET) samples is a daily routine in molecular pathology laboratories, but extraction from FFPET is not always easily achieved. Choosing the right extraction technique is key for further examinations. Several commercial kits are available on the molecular biology market, including both manual isolation procedures and automated extraction. When choosing the right method for isolation, consideration must be given to the aspects of time, precision, downstream applications and price. Choosing the right technique is key for success in molecular biology, because nucleic acid isolation is always the first step in molecular biology and molecular pathology.

Research motivation

The aim of this paper was to compare the advantages and disadvantages of four DNA extraction kits used in daily practice for DNA isolation from formalin-fixed, paraffin-embedded (FFPE) colorectal adenocarcinoma (CRC) surgical specimens: two manual and two automated magnetic bead kits. A correlation of the results with the clinicopathological features of CRCs was also performed.

Research objectives

By comparing the advantages and disadvantages of nucleic acid isolation techniques used in daily routines, precise decisions can be made regarding the most suitable DNA extraction approach for molecular applications.

Research methods

DNA was extracted from FFPE-CRCs. The selection of tumor area was based on the presence of tumor cells in over 80% of the marked tissue, without necroses, hemorrhages, inflammatory, or highly fibrotic stroma. For manual DNA isolation, two commercially available kits were used: The PureLink Genomic DNA Mini Kit from Invitrogen Carlsbad, CA 92008, United States and the High Pure FFPE DNA Isolation Kit from Roche GmbH, Mannheim, Germany. For automated DNA isolation, two commercially available automated magnetic bead kits were used: The iPrep Genomic DNA Kit from Invitrogen and the MagnaPure LC DNA Isolation Kit from Roche. DNA parameters (concentration and quality) were determined using a Nanodrop machine (ThermoScientific, United States). Readings were taken at wavelengths of 260 nm and 280 nm. The optical density (OD) ratio (A260/A280) was automatically calculated, before being correlated with tumor localization, macroscopic and microscopic features, the depth of infiltration, the lymph node ratio, and tumor stage, which were determined according to the latest classification rules.

Research results

DNA concentration was influenced by the macroscopic features and grade of differentiation. A higher DNA concentration was obtained for polypoid compared with ulcero-infiltrative carcinomas, with both Roche systems and using the automated system from Invitrogen. The manual kit from Invitrogen allowed good concentrations to be extracted, but in half of the cases (23 of 46 cases) a value below 150 ng/μL was obtained. For this reason, the *P* value was considered to be at the limit of statistical significance.

Research conclusions

Manual methods of DNA extraction are more controllable and allow the in-house adaptation of the protocol. The obtained DNA concentrations and purity are higher. Automated methods are a time-saving option for polymerase chain reaction (PCR) and real-time quantitative PCR reactions. For CRC samples, a higher DNA concentration is expected to be obtained from differentiated polypoid carcinomas.

Research perspectives

DNA integrity is higher when manual purification is performed, for both tissues and whole blood. The unresolved issue refers to the imbalance between concentration and quality. The above-mentioned aspects should be investigated in larger cohorts with sample size calculations.

REFERENCES

1 **Dahm R**. Discovering DNA: Friedrich Miescher and the early years of nucleic acid research. *Hum Genet* 2008; **122**: 565-581 [PMID: 17901982 DOI: 10.1007/s00439-007-0433-0]

2 **Miller SA**, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215 [PMID: 3344216 DOI: 10.1093/nar/16.3.1215]

3 **Lahiri DK**, Nurnberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 1991; **19**: 5444 [PMID: 1681511 DOI: 10.1093/nar/19.19.5444]

4 **Goelz SE**, Hamilton SR, Vogelstein B. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. *Biochem Biophys Res Commun* 1985; **130**: 118-126 [PMID: 2992457 DOI: 10.1016/0006-291X(85)90390-0]

5 **Ludyga N**, Grünwald B, Azimzadeh O, Englert S, Höfler H, Tapio S, Aubele M. Nucleic acids from long-term preserved FFPE tissues are suitable for downstream analyses. *Virchows Arch* 2012; **460**: 131-140 [PMID: 22270699 DOI: 10.1007/s00428-011-1184-9]

6 **Barresi V**, Cinnirella G, Valenti G, Spampinato G, Musso N, Castorina S, Condorelli DF. Gene

expression profiles in genome instability-based classes of colorectal cancer. *BMC Cancer* 2018; **18**: 1265 [PMID: 30563495 DOI: 10.1186/s12885-018-5174-z]
7 **Smith C**, Otto P, Bitner R, Shiels G. A silica membrane-based method for the isolation of genomic DNA from tissues and cultured cells. *CSH Protoc* 2006; **2006** [PMID: 22485501 DOI: 10.1101/pdb.prot4097]
8 **Fulop ZZ**, Gurzu S, Bara T, Dragus E, Bara T, Voidazan S, Banias L, Jung I. Lymph node ratio, an independent prognostic factor for patients with stage II-III rectal carcinoma. *Pathol Res Pract* 2019; **215**: 152384 [PMID: 30910253 DOI: 10.1016/j.prp.2019.03.013]
9 **Jordan P**. Colorectal Cancer Subtypes - The Current Portrait. *Adv Exp Med Biol* 2018; **1110**: 1-6 [PMID: 30623362 DOI: 10.1007/978-3-030-02771-1_1]
10 **Tan SC**, Yiap BC. DNA, RNA, and protein extraction: the past and the present. *J Biomed Biotechnol* 2009; **2009**: 574398 [PMID: 20011662 DOI: 10.1155/2009/574398]
11 **Hennig G**, Gehrmann M, Stropp U, Brauch H, Fritz P, Eichelbaum M, Schwab M, Schroth W. Automated extraction of DNA and RNA from a single formalin-fixed paraffin-embedded tissue section for analysis of both single-nucleotide polymorphisms and mRNA expression. *Clin Chem* 2010; **56**: 1845-1853 [PMID: 20947696 DOI: 10.1373/clinchem.2010.151233]
12 **Bohmann K**, Hennig G, Rogel U, Poremba C, Mueller BM, Fritz P, Stoerkel S, Schaefer KL. RNA extraction from archival formalin-fixed paraffin-embedded tissue: a comparison of manual, semiautomated, and fully automated purification methods. *Clin Chem* 2009; **55**: 1719-1727 [PMID: 19617290 DOI: 10.1373/clinchem.2008.122572]
13 **Sengüven B**, Baris E, Oygur T, Berktas M. Comparison of methods for the extraction of DNA from formalin-fixed, paraffin-embedded archival tissues. *Int J Med Sci* 2014; **11**: 494-499 [PMID: 24688314 DOI: 10.7150/ijms.8842]
14 **Kocjan BJ**, Hošnjak L, Poljak M. Commercially available kits for manual and automatic extraction of nucleic acids from formalin-fixed, paraffin-embedded (FFPE) tissues. *Acta Dermatovenerol Alp Pannonica Adriat* 2015; **24**: 47-53 [DOI: 10.15570/actaapa.2015.12]
15 **Kalmár A**, Péterfia B, Wichmann B, Patai ÁV, Barták BK, Nagy ZB, Furi I, Tulassay Z, Molnár B. Comparison of Automated and Manual DNA Isolation Methods for DNA Methylation Analysis of Biopsy, Fresh Frozen, and Formalin-Fixed, Paraffin-Embedded Colorectal Cancer Samples. *J Lab Autom* 2015; **20**: 642-651 [PMID: 25576093 DOI: 10.1177/2211068214565903]
16 **Riemann K**, Adamzik M, Frauenrath S, Egensperger R, Schmid KW, Brockmeyer NH, Siffert W. Comparison of manual and automated nucleic acid extraction from whole-blood samples. *J Clin Lab Anal* 2007; **21**: 244-248 [PMID: 17621359 DOI: 10.1002/jcla.20174]

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