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Original research

Molecular analysis of the APC gene in Sicilian patients with familial adenomatous polyposis (F.A.P.)

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ABSTRACT

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited syndrome, caused by germline mutations in the adenomatous polyposis coli (APC) suppressor gene. Patients with colorectal polyps are more likely to develop a malignant condition with poor prognosis. Typical FAP is characterized by hundreds to thousands of colorectal adenomatous polyps and by several extra-colonic manifestations; an attenuated form of polyposis (AFAP), presenting less than 100 adenomas and later onset, has been reported.

In this study we have examined five Sicilian families affected by FAP syndrome, in order to provide predictive genetic testing for the affected families, as well as to contribute to mutation catalog enrichment.

We have detected different APC mutations in these five pedigrees, confirming the remarkable heterogeneity of the mutational spectrum in FAP.

1. Introduction

Familial adenomatous polyposis (FAP) (MIM 175100) is an autosomal-dominant precancerous condition characterized by the appearance of hundreds to thousands of adenomatous polyps throughout the entire colorectum that may result in colorectal cancers [1].

Furthermore, FAP can be characterized by the presence of polyps and adenomas in the stomach and duodenum, desmoid tumors (infiltrative fibromatosis), congenital hypertrophy of the retinal pigment epithelium, osteomas and sebaceous cysts (epidermoid cysts), epatoblastoma, fundic gland polyposis, pancreatic and thyroid cancers, dental abnormalities and malignant of the central nervous system [2–5].

APC is a tumor suppressor gene located on the long arm of the chromosome 5 in band q21 (5q21). The coding region is divided into 15 exons (with a particularly large 15th exon that cover about 76% of the coding region with its 6774 bp) and with its 8529 bp open reading frame encodes a large protein (309 kilo-Daltons) composed of 2843 amino acids [6,7]

The APC protein has multiple domains that mediate oligomerization as well as binding to a variety of intercellular proteins, which have an important role in cell adhesion, signal transduction and transcriptional activation.

Loss of normal APC function is known to be an early event in both familiar and sporadic colon cancer pathogenesis occurring at the preadenoma stage [8]

Mutations of the APC gene have been implicated in familial adenomatous polyposis as well as sporadic colorectal tumors, as they are involved in the early stage of colorectal tumorigenesis.

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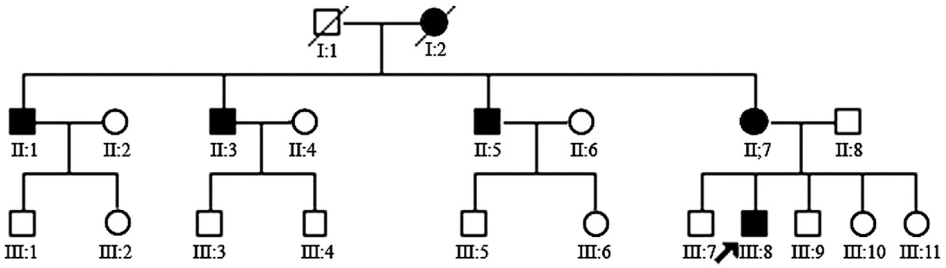

Fig. 1. Family n.1 tree.

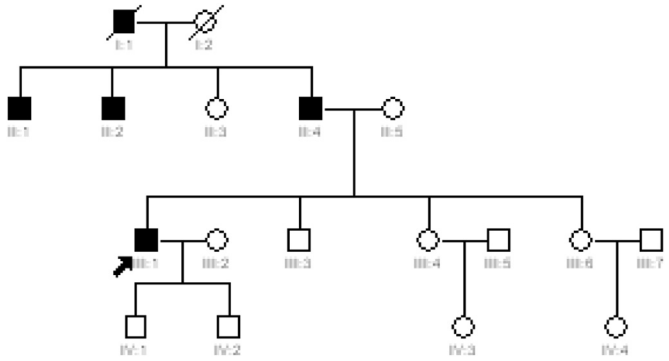

Fig. 2. Family n.2 tree.

Most of the germline mutations identified in FAP patients result in a truncated protein due to a premature stop codon or frame shifts.

About 60% of the *APC* mutations in sporadic colorectal tumors are clustered in the central domain of *APC* (amino acids 1284–1580), also called mutation cluster region (MCR) [9]. In addition to the tumor suppressor function of colorectal tumors, APC protein has been demonstrated to possess many other functions, including regulation of cell proliferation, differentiation and migration [10].

Considering the high grade of penetrance in FAP mutations carriers and the high relevance of genetic diagnostic test for early detection and prevention of cancer, we decided to performed a screening of the APC gene in patients with FAP syndrome.

In this paper we report the identification of germline *APC* mutations in five unrelated FAP Sicilian families.

2. Materials and methods

2.1. Patients

All patients with Sicilian origin were recruited through cancer centers and gastroenterology services in Sicilian medical institutions. Probands have been initially identified by the presence of more than 100 colorectal polyps on endoscopic examination and/or by a family history of FAP.

In our patients or their relatives no other lesions associated with the polyposis were found, except for a member of FAM4 (III:1), who presented desmoid tumors.

Among members of FAP families, several adenocarcinomas have been diagnosed before 40 years of age. Genetic analysis of each family was included at least one symptomatic case.

Informed consent was obtained from all participants before testing. Genealogical trees of all families were prepared during genetic counseling (Figs. 1–5).

FAMILY 1: family history was positive for FAP and colic tumors (I:2, II:1, II:3, II:5, II:7). The proband III:8, underwent endoscopic examination at the age of 10 for suspicion of FAP, but no polyps were found. Five years later a new endoscopic examination revealed several colic polyps. Since then the patient has been subjected topolypectomy in different endoscopic sessions.
FAMILY 2: the proband (III:1) at the age of 37 years was diagnosed with FAP syndrome and colectomized. Other family members, I:1, II:1, II:2, II:4, III:4 and III:6 were subjected to total colectomy for polyposis and other colic tumors.
FAMILY 3: the proband (II:1) was colectomized for FAP at the age of 45. His father (I:1) died for colon cancer and his sister (II:3) was colectomized for FAP at the age of 40.
FAMILY 4: the proband (III:3)was colectomized for diffuse polyposis at the age of 20. Her mother (II:1) died for colon cancer at the age of 40 and her brother (III:1), who presented desmoid tumors, was colectomized for Gardner's Syndrome at the age of 18; uncles (II:3, II:4, II:5, II:7) were also affected by FAP syndrome.
FAMILY 5: the proband (III:1) was colectomized for colon cancer, associated with diffuse polyposis, at the age of 38. Subjects II:1, II:3 and III:6 were referred to as being affected of polyposis and

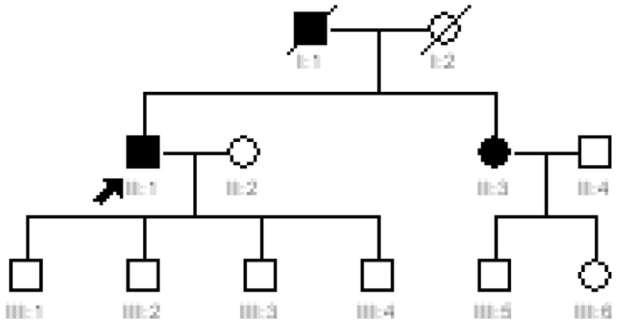

Fig. 3. Family n.3 tree.

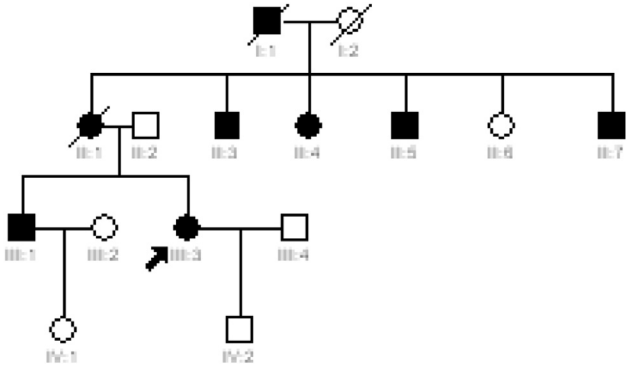

Fig. 4. Family n.4 tree.

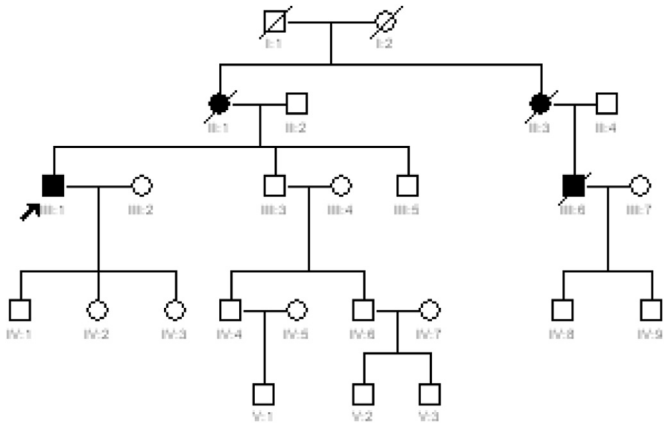

Fig. 5. Family n.5 tree.

dead for colon cancer. Fourth-generation and fifth-generation individuals asked to attend colonoscopy following mutation identification.

3. Methods

3.1. DNA extraction and PCR amplification

After informed consent, blood samples were obtained from affected individuals. Genomic DNA was purified by the phenol-chloroform method and stored at −20 °C.

Mutation analysis of APC gene was performed using PCR amplification covering coding sequences and intron–exon boundaries of exons 1 to 14 and segments of exon 15 was performed using the primers reported elsewhere [6].

Amplification products were run by 2% agarose gel electrophoresis stained with SYBR Safe DNA gel stain.

3.2. Direct sequencing of PCR-amplified fragments

Direct sequencing of amplified products was performed to determine sequence changes. Each amplified product was purified by a Montage PCR Centrifugal Filter (Millipore) to remove unincorporated precursors. The purified fragments were then sequenced by using the fluorescent BigDye Terminator Chemistry (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol and analyzed by using a DNA automated ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The results of sequencing analysis were compared with wild-type samples and the normal sequencing of the APC gene (OMIM 175100; Gen Bank: 4557318). Mutations were confirmed by two independent PCR reactions and by sequencing both strands.

Table 1
APC mutations found in proband's families.

Family ID	Codon	Exon	Mutation	Mutation type	Consequence
FAM 1	1062	15	c.3186-3187delAA	Frameshift	Chain ter
FAM 2	1981	15	c.5942delA	Frameshift	Chain ter
FAM 3	1104	15	c.3311C>A (S1104X)	Nonsense	Chain ter
FAM 4	1309	15	c.3927-3931delAAAGA	Frameshift	Chain ter
FAM 5	624	14	c.1875-1878delGACA	Frameshift	Chain ter

Table 2
Family members showing the specific gene alteration after genetic counseling.

Family ID	Proband	Positive relatives
FAM1	III:8	III:1, III:3, III:7
FAM2	III:1	IV:1
FAM3	II:1	II:3, III:1, III:3, III:6
FAM4	III:3	–
FAM5	III:1	IV:2, IV:9, V:1

4. Results

In Table 1 are reported the different APC mutations found in proband's families, four deletions and a single substitution. Mutations are named according to the nomenclature proposed by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/).

In the five informative families the germinal mutation was confirmed in several family members at risk. These individuals had not previously been analyzed and were all asymptomatic.

In Table 2 are described all family members showing the specific gene alteration after genetic counseling.

5. Discussion

Truncating germline mutation in the APC gene are responsible for 70–90% of FAP cases [11].

Only one study clearly reports a characterized APC mutation from a Sicilian family with FAP [12].

All the mutations of the *APC* gene, found in our probands, cause the introduction of premature stop codons and consequent formation of a truncated protein.

The first deletion, found in the FAM1, results in the loss of two bases (−AA) in codon 1062. Although rarely reported [13] this deletion overlaps with a mutational hot-spot of the FAP syndrome, codons 1062 and 1063, and involves the binding site for beta-catenin [14]. Interestingly, we learned that the individual, examined in Gismondi's study and carrying the same deletion, was of Sicilian origin. This observation suggests that this mutation may be recurrent in the Sicilian population even if larger number of individuals affected by FAP are needed to evaluate the prevalence of such mutation.

The second deletion, found in the FAM2, results in the loss of one single base (−A), in codon 1981, as previously described by Scott et al. [15].

In the FAM4, we found another deletion, which consists in the loss of 5 bp (−AAAGA) in codon 1309, as previously described by Miyoshi et al. [16]. This deletion falls within a region where the mutation has been shown to have a strong correlation with one of the features of the Gardner's syndrome such as the presence of desmoids, observed in the proband's brother. A fourth deletion was found in the FAM5, and results in the loss of 4 bp (−GACA) in codon 624 as previously described by Nagase et al. [17].

FAM3 was characterized by the mutation c.3311C>A, that results in a truncated APC protein causing the FAP phenotype [18]. It consists in a substitution (TCA > TAA) in codon 1104 causing a stop-codon, in position of serine.

This mutation falls in the region of exon 15, comprised between codons 876 and 1344, that correlates with extra-colic adenomas manifestation and the hypertrophy of the retinal pigment epithelium (CHRPE) phenotype. It is interesting to notice that the proband (II:1) and her sister (II:3) manifested retinal anomalies and epidermoid cysts on the back.

The diagnosis of FAP, in probands and in the majority of their relatives, was made after finding colonic polyps in colonoscopy. For

some of them, it was not possible to perform the colonoscopy because of the young age or patient refusal to have endoscopic interventions [19–21].

In conclusion, most of APC gene alterations here reported are small deletions previously described in other FAP individuals. Interestingly, the present study allow us to identify a new mutation in a Sicilian family with FAP history.

We believe that enlargement of the APC mutation spectrum with these types of studies will contribute to early detection of patients with FAP from different populations and to the prevention of colorectal cancer formation [22–29].

Ethical approval

This is a retrospective study based only on the analyses of recorded data and then no Ethical Approval was necessary.

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Author contribution

Angela Russo: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Vito Emanuele Catania: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Bartolomea Ficili: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Eleonora Lanteri: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Paolo Tiralongo: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Andrea Cavallaro: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Corrado Randazzo: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Fernando Cammisuli: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Roberto Madeddu: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Vincenzo Trichilo: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Massimo Libra: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Salvatore Travali: Participated substantially in conception, design, and execution of the study and in the analysis and interpretation of data; also participated substantially in the drafting and editing of the manuscript.

Conflicts of interest

All Authors have no conflict of interests.

References

[1] N.S. Feamhead, M.P. Britton, W.F. Bodmer, The ABC of APC, Hum. Mol. Genet. 10 (2001) 721–733.
[2] M. Nilbert, U. Kristoffersson, M. Ericsson, O. Johannsson, E. Rambech, P. Mangell, Broad phenotypic spectrum in familial adenomatous polyposis; from early onset and severe phenotypes to late onset of attenuated polyposis with the first manifestation at age 72, BMC Med. Genet. 9 (2008 Nov 26) 101.
[3] C. Soravia, T. Berk, Z. Cohen, Genetic testing and surgical decision making in hereditary colorectal cancer, Int. J. Colorectal Dis. 15 (1) (2000 Feb) 21–28.
[4] C. Soravia, T. Berk, R.S. McLeod, Z. Cohen, Desmoid disease in patients with familial adenomatous polyposis, Dis. Colon Rectum 43 (3) (2000 Mar) 363–369.
[5] J. Church, C. Lynch, P. Neary, L. LaGuardia, E. Elayi, A desmoid tumor-staging system separates patients with intra-abdominal, familial adenomatous polyposis-associated desmoid disease by behavior and prognosis, Dis. Colon Rectum 51 (6) (2008 Jun) 897–901.
[6] J. Groden, A. Thliveris, W. Samowitz, M. Carlson, L. Gelbert, H. Albertsen, G. Joslyn, J. Stevens, L. Spirio, M. Robertson, et al., Identification and characterization of the familial adenomatous polyposis coli gene, Cell 66 (3) (1991 Aug 9) 589–600.
[7] K.W. Kinzler, M.C. Nilbert, L.K. Su, et al., Identification of FAP locus genes from chromosome 5q21, Science 253 (1991) 661–664.
[8] S.M. Powell, N. Zilz, Y. Beazer-Barclay, T.M. Bryan, S.R. Hamilton, S.N. Thibodeau, B. Vogelstein, K.W. Kinzler, APC mutations occur early during colorectal tumorigenesis, Nature 359 (1992) 235–237.
[9] Y. Miyoshi, H. Nagase, H. Ando, A. Horii, S. Ichii, S. Nakatsuru, T. Aoki, Y. Miki, T. Mori, Y. Nakamura, Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene, Hum. Mol. Genet. 1 (1992) 229–233.
[10] M. Mihalatos, I. Danielides, J. Belogianni, et al., Novel mutations of the APC gene in familial adenomatous polyposis in Greek patients, Cancer Genet. Cytogenet 141 (2003) 65–70.
[11] L. Lipton, I. Tomlinson, The genetics of FAP and FAP-like syndromes, Fam. Cancer 5 (2006) 221–226.
[12] R. Terranova, S. Luca, L. Salmieri, About a case of familial adenomatous polyposis, Minerva Gastroenterol. Dietol. 45 (1999) 271–281.
[13] W. Friedl, S. Aretz, Familial adenomatous polyposis: experience from a study of 1164 unrelated German polyposis patients, Hered. Cancer Clin. Pract. 3 (2005) 95–114.
[14] V. Gismondi, A. Bafico, R. Biticchi, S. Pedemonte, F. Molina, A. Heouaine, P. Sala, L. Bertario, S. Preciuttini, P. Stringini, J. Groden, L. Varesco, Characterization of 19 novel and six recurring APC mutations in Italian adenomatous polyposis patients, using two different mutation detection techniques, Hum. Mutat. 9 (1997).
[15] R.J. Scott, R. van der Luijt, M. Spycher, J.L. Mary, A. Muller, T. Hoppeler, M. Haner, H. Muller, S. Martinoli, P.L. Brazzola, Novel germline APC gene mutation in a large familial adenomatous polyposis kindred displaying variable phenotypes, Gut 38 (1996).
[16] Y. Myoishi, H. Ando, H. Nagase, I. Nishisho, A. Horii, Y. Miki, T. Mori, J. Utsunomiya, S. Baba, G. Petersen, S.R. Hamilton, K.W. Kinzler, Y. Vogelstein Band Nakamura, Germ-line mutation of the APC gene in 53 familial adenomatous polyposis patients, Proc. Natl. Acad. Sci. U. S. A. 89 (1992).
[17] H. Nagase, Y. Nakamura, Mutation of the APC (Adenomatous polyposis coli) gene, Hum. Mutat. 2 (1993) 425–434.
[18] B. Rivera, S. González, E. Sánchez-Tomé, I. Blanco, F. Mercadillo, R. Letón, J. Benítez, M. Robledo, G. Capellá, M. Urioste, Clinical and genetic characterization of classical forms of familial adenomatous polyposis: a Spanish population study, Ann. Oncol. 22 (2011) 903–909.
[19] M. Malaguarnera, M. Vacante, G. Condorelli, et al., Probiotics and prebiotics in the management of constipation in the elderly, Acta Med. Mediterr. 29 (2013) 791–798.
[20] G. Malaguarnera, I. Paladina, M. Giordano, M. Malaguarnera, G. Bertino, M. Berretta, Serum markers of intrahepatic cholangiocarcinoma, Dis. Markers 34 (4) (2013) 219–228.
[21] M. Uccello, G. Malaguarnera, F. Basile, V. D'agata, M. Malaguarnera, G. Bertino, M. Vacante, F. Drago, A. Biondi, Potential role of probiotics on colorectal cancer prevention, BMC Surg. 12 (Suppl. 1) (2012) S35.
[22] M. Ragusa, L. Statello, M. Maugeri, A. Majorana, D. Barbagallo, L. Salito, M. Sammito, M. Santonocito, R. Angelica, A. Cavallaro, M. Scalia, R. Caltabiano, G. Privitera, A. Biondi, M. Di Vita, A. Cappellani, E. Vasquez, S. Lanzafame,

E. Tendi, S. Celeste, C. Di Pietro, F. Basile, M. Purrello, Specific alterations of the microRNA transcriptome and global network structure in colorectal cancer after treatment with MAPK/ERK inhibitors, J. Mol. Med. Berl. 90 (12) (2012 Dec) 1421–1438.

[23] A. Cappellani, A. Zanghi, M. Di Vita, E. Zanet, P. Veroux, B. Cacopardo, A. Cavallaro, G. Piccolo, E. Lo Menzo, P. Murabito, M. Berretta, Clinical and biological markers in gastric cancer: update and perspectives, Front. Biosci. Sch. Ed. 2 (2010 Jan 1) 403–412.

[24] A. Cappellani, M. Di Vita, A. Zanghi, P. Veroux, A. Cavallaro, E. Lo Menzo, B. Cacopardo, V. Canzonieri, P. Murabito, U. Tirelli, M. Berretta, Biological and clinical markers in colorectal cancer: state of the art, Front. Biosci. Sch. Ed. 2 (2010 Jan 1) 422–431.

[25] G. Ligresti, L. Militello, L.S. Steelman, A. Cavallaro, F. Basile, F. Nicoletti, F. Stivala, J.A. McCubrey, M. Libra, PIK3CA mutations in human solid tumors: role in sensitivity to various therapeutic approaches, Cell Cycle 8 (9) (2009 May 1) 1352–1358.

[26] M. Malaguarnera, E. Cristaldi, L. Cammalleri, V. Colonna, H. Lipari, A. Capici, A. Cavallaro, M. Beretta, I. Alessandria, S. Luca, M. Motta, Elevated chromogranin A (CgA) serum levels in the patients with advanced pancreatic cancer, Arch. Gerontol. Geriatr. 48 (2) (2009) 213–217.

[27] A. Cavallaro, A. Lauretta, S. Pennisi, D. Di Mauro, M. Cavallaro, Hereditary non poliposys colorectal cancer (HNPCC) prevention, Chirurgia 18 (5) (2005) 263–268.

[28] A. Plawski, T. Banasiewicz, P. Borun, L. Kubaszewski, P. Krokowicz, M. Skrzypczak-Zielinska, J. Lubinski, Familial adenomatous polyposis of the colon, Hered. Cancer Clin. Pract. 11 (1) (2013 Oct 22) 15.

[29] B.A. Talseth-Palmer, J.T. Wijnen, E.K. Andreassen, D. Barker, S. Jagmohan-Changur, C.M. Tops, C. Meldrum, A. Spigelman, F.J. Hes, T. Van Wezel, H.F. Vasen, R.J. Scott, Dutch Cancer Genetics Group, The importance of a large sample cohort for studies on modifier genes influencing disease severity in FAP patients, Hered. Cancer Clin. Pract. 11 (1) (2013 Dec 29) 20.