Receptors & Clinical Investigation 2015; 2: e635. doi: 10.14800/rci.635; © 2015 by Ana M. Ferreira, *et al.*
http://www.smartscitech.com/index.php/rci

REVIEW

Estrogen pathway mutations and cancer

Ana M. Ferreira[1,2], Robert M.W. Hofstra[3], Helga Westers[4]

[1]*Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Évora 7006-554, Portugal*
[2]*Plant Cell Biotechnology Lab, Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras 2780-157, Portugal.*
[3]*Department of Clinical Genetics, Erasmus MC, 3015 CE Rotterdam, the Netherlands*
[4]*Department of Genetics, University Medical Center Groningen, University of Groningen, 9700 RB Groningen, the Netherlands*

Correspondence: Dr. H. Westers
E-mail: h.westers@umcg.nl
Received: February 12, 2015
Published online: April 02, 2015

Cancer is caused by an accumulation of mutations in a stem cell. A defective mismatch repair (MMR) system can lead to such an accumulation of mutations. MMR defects are found in a cancer syndrome called Lynch Syndrome, and tumors of this syndrome are indeed characterized by such an accumulation of mutations, particularly in short repetitive DNA sequences, called microsatellites. When such mutated microsatellites are located in the coding sequences of genes with essential roles for tumorigenesis, we speak of 'target genes'. Many such target genes have been found and in this review we focus the possible involvement of target genes involved in the estrogen-receptor pathway (ER). We recently identified *NRIP1*, encoding the nuclear receptor-interacting protein 1, as the most frequently mutated gene in microsatellite instable (MSI) endometrial cancer (EC). NRIP1 is a known corepressor of the ER pathway, the pathway essential in regulating the concentrations of estrogens, a hormone for which the endometrium is highly responsive. This in combination with the fact that high exposure to estrogens is currently considered the major risk factor for EC - approximately 80% of all sporadic EC tumors are estrogen dependent carcinomas - make NRIP1 the perfect target gene for EC. Interestingly, mutations in *NRIP1* were also detected in MSI colorectal carcinoma (CRC) samples. Finding mutations in an estrogen receptor signaling protein in colorectal tissue might not be that expected, as colon is not typically associated with being responsive to estrogens. However, evidence is accumulating to better understand this finding. For instance, it was shown that *NRIP1*, in colon tissue, stimulates *APC* gene transcription and inhibits β-catenin activation. Moreover, some studies suggested that estrogens can increase the expression of *MLH1* in colon cancer cells, highlighting the implications of estrogen protecting against colon cancer, by regulating the MMR system. All in all we conclude that genes involved in the estrogen pathway are the perfect candidates to be studied in MMR-deficient tumors, especially those developing in hormonal responsive tissues.

To cite this article: Ana M. Ferreira, *et al*. Estrogen pathway mutations and cancer. Receptor Clin Invest 2015; 2: e635. doi: 10.14800/rci.635.

Mismatch repair (MMR) and endometrial carcinomas

Genetic inactivation of mismatch repair (MMR) genes, by mutations or epigenetic alterations, eventually leads to the accumulation of mutations that otherwise would be corrected. These mutations are easily visualized by analyzing short repetitive DNA sequences, called microsatellites. An accumulation of mutations in such microsatellites is known as microsatellite instability or MSI. MSI, caused by defects in the MMR system, is the hallmark of Lynch syndrome, a hereditary disease, characterized by the development of hereditary carcinomas, such as colorectal-, endometrial-, and gastric carcinomas. Moreover MSI is also observed in a percentage (15-25%) of sporadic tumors [1-5].

Most microsatellites are found outside genes. However, they can also occur within coding and non-coding parts of genes. When mutations occur in such coding intragenic microsatellites they often are out of frame mutations which can lead to nonfunctional, foreign proteins. As mutations occur at random they mostly occur in genes not involved in the cancer developmental process - these genes are called 'bystander genes'. However, they also can hit genes with essential roles for tumorigenesis. These are called 'target genes'. Over 160 of genes have been identified and studied in MMR-deficient tumors of several tissue origins [6-8]. Most searches for target gene mutations were done in MSI colorectal carcinomas (CRC). Mutation screens in other tumor types were mostly performed by studying target genes previously detected in colorectal tumors. Therefore, specific tumorigenesis pathways associated to a specific type of tissue might have been missed using this strategy. That was the reason why we performed an unbiased screen for target gene mutations in endometrial carcinomas [9].

Target genes in endometrial carcinomas

To perform this unbiased study we first determined which genes normally are expressed in endometrial tissue. Therefore, we performed expression profiling experiments on freshly-frozen normal endometrial tissue samples. We selected 2,338 genes showing expression values 10 times higher than the background signals. These genes were subsequently screened for the presence of a microsatellite, in particular mononucleotide tracts of (A)7, (T)7, (C)7, (G)7, (A)8, and (T)8 in their coding sequences. Repeat lengths were chosen according to the paper of Sammalkorpi *et al.* (2007) [10]. A total of 482 genes with such repeats were identified and selected for mutation screening of their coding DNA microsatellites in a set of 42 MSI-High endometrial tumor samples. Doing so we identified 44 possible target genes of which seven are highly mutated (>15%). Some candidates were also found mutated in colorectal and gastric tumors [9].

NRIP1 the perfect target gene for endometrial cancer

The most promising gene coming out of this screen, based on mutation frequency and function, is *NRIP1*. *NRIP1* encodes for the nuclear receptor-interacting protein 1. *NRIP1* was mutated in 34% of the tumor samples analyzed and is as such the most frequently mutated gene in MSI endometrial cancer. Functionally, we also can very well link NRIP1 to the development of endometrial cancer: NRIP1 is a known corepressor of the estrogen-receptor (ER) pathway, the pathway essential in regulating the concentrations of estrogens, a hormone for which the endometrium is highly responsive. This in combination with the fact that high exposure to estrogens is currently considered the major risk factor for EC - approximately 80% of all sporadic EC tumors are estrogen-dependent carcinomas - strongly point out *NRIP1* as the perfect target gene for EC.

Considering tumorigenesis of other hormone-dependent tissue types, *NRIP1* is also for these the perfect target gene as it is generally accepted that genetic alterations in ER and in ER responsive genes likely are key players in the development of hormone-dependent tumors [11]. More specifically, Ghoussaini [12] suggested the importance of *NRIP1* for estrogen-responsive tumors, and in fact more studies of *NRIP1* in breast and ovarian tissues, for instance, came out in the last years [12,13].

NRIP1 silencing in endometrial cancer

We studied the effect of losing this gene in the context of a MSI endometrial cancer: we silenced the *NRIP1* gene in the HEC-1B cell line, a human MSI endometrial epithelial cancer cell line, and performed an expression microarray. Subsequently, network analysis of the respective expression results was performed. We observed that silencing of *NRIP1* is accompanied by differences in the expression of the genes of the ER network. Also the RAR pathway was one of the networks showing a deregulation of many of its members due to *NRIP1* knock-down. On forehand we expected that the inactivation of *NRIP1* would interfere with the process of co-repression of the ER complex and lead to differences in the expression of estrogen-dependent genes; indeed that's what the Ingenuity Pathway analysis (IPA) showed us [9].

From literature it is known that there is an important correlation between NRIP1 and the RAR pathways, and that the RAR pathway is implicated in many types of cancer, including endometrial cancer and other hormone-dependent cancers [14-17]. Our results from the expression microarrays, Ingenuity Pathway Analysis (IPA), **Gene Set Enrichment Analysis** (GSEA), and RT-PCR analyses add to this line of reasoning, that *NRIP1* likely is an essential gene in tumorigenesis of MSI endometrial tumors when inactivated in some way. Moreover it suggests NRIP1 to be a key player in the cross-talk between ER and RAR in this set of tumors, as we observe expression alterations in genes, such as *NCOR2*, simultaneously belonging to ER and RAR pathways (Supplementary Figure 1).

***NRIP1* and colorectal carcinomas**

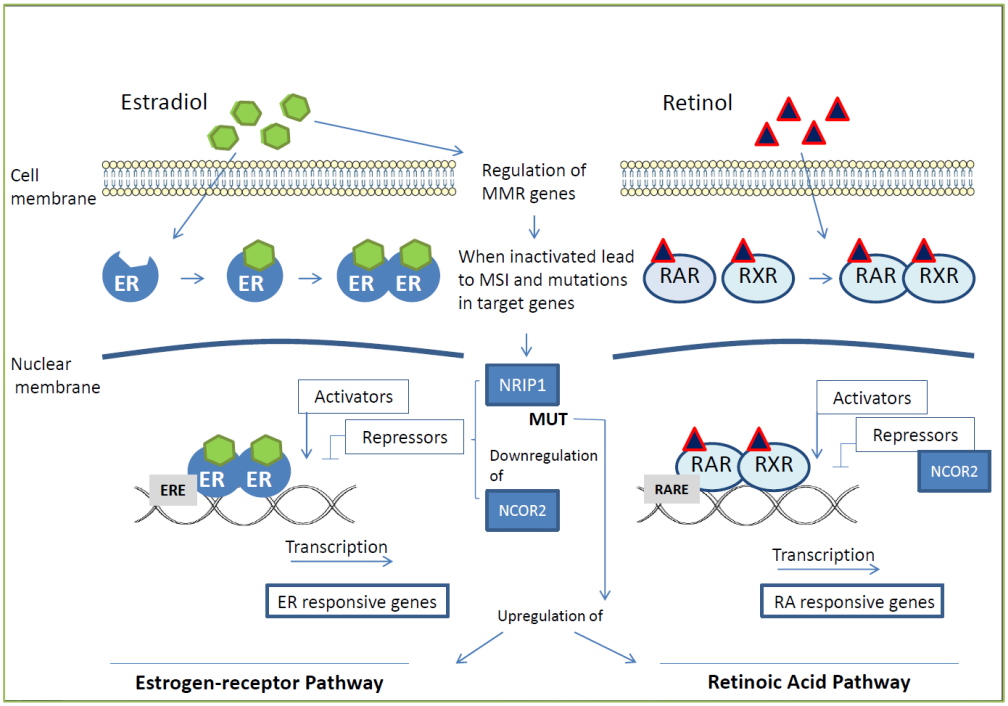

Figure 1. Estrogen-receptor and retinoic acid pathways, showing the role and interplay of the nuclear repressors, such as NRIP1 and NCOR2, on transcription. The influence of estrogens on MMR is also represented. ER: estrogen receptor; MMR: mismatch repair; MUT: mutation; RAR: retinoic acid receptor; RXR: retinoid X receptor; ERE: estrogen response elements; RARE: retinoic acid response elements.

The target genes profile of EC and gastrointestinal tumors with MMR deficiency differs, both in a qualitative and in a quantitative way [18-20]. However, there are target genes commonly involved in MSI-High tumors of diverse origin. As for *NRIP1*, in the same study we detected a mutation frequency of 22% for MSI colorectal carcinoma (CRC) samples and 13% for MSI gastric carcinoma samples. The mutation frequency in gastric carcinomas might suggest a low impact of the *NRIP1* mutation on tumor development; however for CRC the mutation frequency is considerably high. Mutations in this gene in CRC have been reported previously, although in a different coding repeat and with different frequency [7]. Our finding, that NRIP1 is also an important player in CRC development was corroborated by a recent study. Using loss and gain of function mouse models, Lapierre *et al.* [21], were able to show that NRIP1 inhibits the proliferation and apoptosis of intestinal epithelial cells. The authors demonstrated that both in mice tissues and in human cancer cell lines, NRIP1 stimulates *APC* gene transcription and inhibits β-catenin activation, this way playing an important role in CRC development.

Finding a gene involved in estrogen receptor signaling in colorectal tissue is not that expected, as colon is not typically associated with being responsive to certain hormones. However, there is a growing perception that estrogens have a protective role against the initiation and progression of CRC [22,23]. This could partly explain the gender bias in CRC incidence as women have a lower CRC incidence when compared to man. Moreover, two studies suggested that estrogens can increase the expression of *hMLH1* in colon cancer cells Colo205, which highlight the implications of estrogen protecting against colon cancer, by regulating the MMR system [24-26].

Estrogens and MMR-deficient tumors

We addressed a possible link between estrogens and MMR associated tumor development already several years ago [27]. At that time, we proposed a model in which estrogens could have a protective effect, however this effect is likely lost if the MMR system has somehow been modulated/deregulated. On the other hand we proposed that high estrogen receptor activity might result in DNA damage events, including methylation aberrations or gene mutations. If any of these events target MMR genes, we will observe MSI and possibly an accumulation of mutations in genes. Genes coding for co-factors or ER signaling could eventually be affected in this cascade of events, which subsequently would modulate ER regulated transcription. These hypotheses were based upon work of Slattery and coworkers (2001) [28], who proposed a model for the way in which estrogens (endogenous, exogenous, and obesity-associated) modify the risk of MSI tumors. These authors also suggested

that at least one of the major MMR genes is estrogen-responsive and that loss of estrogens results in loss of DNA mismatch repair capacity. Also Wada-Hiraike *et al*. (2005) [29] defended the hypothesis that common co-activators of ER, and even ER itself, might have a functional role in DNA MMR, and more specifically, they suggested MSH2 as a potent co-activator of ERα.

Nowadays we know that the regulation of estrogen-responsive genes is tissue dependent and that co-activator and co-repressor molecules exist in different combinations and or concentrations, all depending on the tissues type and that all this leads to different results. Anyhow, if we consider that the MMR system is a (direct or indirect) target of estrogens [24,25,28-30], it is reasonable to hypothesize, also in light of the new findings of the NRIP1 studies described here, that genes involved in the estrogen pathway are the perfect candidates to be studied in MMR-deficient tumors, especially those developing in hormonal responsive tissues. In the particular case of NRIP1, there is growing evidence of the importance of this protein in several cellular mechanisms, from development to inflammation and metabolism [31], so we suggest that further studying it and its direct interacting proteins might bring us new insights on their impact in cancer and other human health related problems.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgements

Authors acknowledge financial support from Fundação para a Ciência e a Tecnologia, Portugal, in the form of postdoctoral grant to A.M. Ferreira (grant ref.: SFRH/BPD/69655/2010).

References

1. Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen L, Mecklin JP, *et al*. Clues to the pathogenesis of familial colorectal cancer. Science 1993; 260:812-816.
2. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993; 363:558-561.
3. Peltomaki P, Lothe RA, Aaltonen LA, Pylkkanen L, Nystrom-Lahti M, Seruca R, *et al*. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. Cancer Res 1993; 53:5853-5855.
4. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science 1993; 260:816-819.
5. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, *et al*. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58:5248-5257.
6. Vilkki S, Launonen V, Karhu A, Sistonen P, Vastrik I, Aaltonen LA. Screening for microsatellite instability target genes in colorectal cancers. J Med Genet 2002; 39:785-789.
7. Røyrvik EC, Ahlquist T, Rognes T, Lothe RA. Slip slidin' away: a duodecennial review of targeted genes in mismatch repair deficient colorectal cancer. Crit Rev Oncog 2007; 13:229-257.
8. Alhopuro P, Sammalkorpi H, Niittymaki I, Bistrom M, Raitila A, Saharinen J, *et al*. Candidate driver genes in microsatellite-unstable colorectal cancer. Int J Cancer 2012; 130:1558-1566.
9. Ferreira AM, Tuominen I, Sousa S, Gerbens F, van Dijk-Bos K, Osinga J, *et al*. New target genes in endometrial tumors show a role for the estrogen-receptor pathway in microsatellite-unstable cancers. Hum Mutat. 2014; 35:1514-23.
10. Sammalkorpi H, Alhopuro P, Lehtonen R, Tuimala J, Mecklin JP, J¨arvinen HJ, *et al*. Background mutation frequency in microsatellite unstable colorectal cancer. Cancer Res 2007; 67:5691-5698.
11. Notarnicola M, Gristina R, Messa C, Cariola F, Fiorente P, Caruso ML, *et al*. Estrogen receptors and microsatellite instability in colorectal carcinoma patients. Cancer Lett 2001; 168:65-70.
12. Ghoussaini M, Fletcher O, Michailidou K, Turnbull C, Schmidt MK, Dicks E, *et al*. Genome-wide association analysis identifies three new breast cancer susceptibility loci. Nat Genet 2012; 44:312-8.
13. Docquier A, Garcia A, Savatier J, Boulahtouf A, Bonnet S, Bellet V, *et al*. Negative regulation of estrogen signaling by ERβ and RIP140 in ovarian cancercells. Mol Endocrinol 2013; 27:1429-41.
14. Clarke N, Germain P, Altucci L, Gronemeyer H. Retinoids: potential in cancer prevention and therapy. Expert Rev Mol Med 2004; 6:1-23.
15. Tanabe K, Utsunomiya H, Tamura M, Niikura H, Takano T, Yoshinaga K, *et al*. Expression of retinoic acid receptors in human endometrial carcinoma. Cancer Sci 2008; 99:267-271.
16. Cheng YH, Utsunomiya H, Pavone ME, Yin P, Bulun SE. Retinoic acid inhibits endometrial cancer cell growth via multiple genomic mechanisms. JMol Endocrinol 2011; 46:139-153.
17. Salvatori L, Ravenna L, Caporuscio F, Principessa L, Coroniti G, Frati L, *et al*. Action of retinoic acid receptor on EGFR gene transactivation and breast cancer cell proliferation: interplay with the estrogen receptor. Biomed Pharmacother 2011; 65:307-312.
18. Myeroff LL, Parsons R, Kim SJ, Hedrick L, Cho KR, Orth K, *et al*. A transforming growth factor beta receptor type II gene mutation common in colon and gastric but rare in endometrial cancers with microsatellite instability. Cancer Res 1995; 55:5545-5547.
19. Gurin CC, Federici MG, Kang L, Boyd J. Causes and consequences of microsatellite instability in endometrial carcinoma. Cancer Res 1999; 59:462-466.

20. Duval A, Reperant M, Compoint A, Seruca R, Ranzani GN, Iacopetta B, *et al*. Target gene mutation profile differs between gastrointestinal and endometrial tumors with mismatch repair deficiency. Cancer Res 2002; 62:1609-1612.

21. Lapierre M, Bonnet S, Bascoul-Mollevi C, Ait-Arsa I, Jalaguier S, Del Rio M, *et al*. RIP140 increases APC expression and controls intestinal homeostasis and tumorigenesis. J Clin Invest. 2014; 124:1899-913.

22. Hartman J, Gustafsson JA. Estrogen receptors in colorectal cancer: goalkeepers, strikers, or bystanders? Cancer Prev Res (Phila) 2010; 3:897-899.

23. Hsu HH, Kuo WW, Ju DT, Yeh YL, Tu CC, Tsai YL, *et al*. Estradiol agonists inhibit human LoVo colorectal-cancer cell proliferation and migration through p53. World J Gastroenterol 2014; 20:16665-73.

24. Jin P, Lu XJ, Sheng JQ, Fu L, Meng XM, Wang X, *et al*. Estrogen stimulates the expression of mismatch repair gene hMLH1 in colonic epithelial cells. Cancer Prev Res (Phila) 2010; 3:910-916.

25. Lu XJ, Yu DL, Wang JX, Pan XL, Jin P, Li SR, *et al*. Effect of estrogen on mismatch repair gene expression in colon cancer cells. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2011; 27:754-756.

26. He YQ, Sheng JQ, Ling XL, Fu L, Jin P, Yen L, *et al*. Estradiol regulates miR-135b and mismatch repair gene expressions via estrogen receptor-β in colorectal cells. Exp Mol Med 2012; 44:723-32.

27. Ferreira AM, Westers H, Albergaria A, Seruca R, Hofstra RM. Estrogens, MSI and Lynch syndrome-associated tumors. Biochim Biophys Acta 2009; 1796:194-200.

28. Slattery ML, Potter JD, Curtin K, Edwards S, Ma KN, Anderson K, *et al*. Estrogens reduce and withdrawal of estrogens increase risk of microsatellite instability-positive colon cancer. Cancer Res. 2001; 61:126-130.

29. Wada-Hiraike O, Yano T, Nei T, Matsumoto Y, Matsumoto Y, Nagasaka K, *et al*. The DNA mismatch repair gene hMSH2 is a potent coactivator of oestrogen receptor alpha. Br. J. Cancer 2005; 92:2286-2291.

30. Miyamoto T, Shiozawa T, Kashima H, Feng YZ, Suzuki A, Kurai M, *et al*. Estrogen up-regulates mismatch repair activity in normal and malignant endometrial glandular cells, Endocrinology 2006; 147:4863-4870.

31. Nautiyal J, Christian M, Parker MG. Distinct functions for RIP140 in development, inflammation, and metabolism. Trends Endocrinol Metab 2013; 24:451-459.