Gene expression of chromosome in the rat Endometrial carcinoma

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Abstract

The BDII rat is more likely to get hormone-dependent endometrial adenocarcinoma because of a gene. It was looked at to learn more about cancer in people. Comparative genome hybridization was used to look at tumors that grew on their own in BDII rats of mixed strains. Most problems were caused by a region on rat chromosome 4 called bands q12–q22 that was amplified. Tissue cultures from 11 endometrial carcinomas were used to check the copy number of 15 genes that are linked to cancer (10 endometrial adenocarcinomas and 1 endometrial squamous cell carcinomas).One person had pancreatic cancer and the other had peritoneal mesothelioma. In six of the tumors, the rat's chromosome 4 was made bigger. Met (hepatocyte growth factor receptor) and Cdk6 (cyclindependent kinase 6) were amplified the most and the most often. Five of the tumors had two amplified areas at 4q12-q13 and 4q21-q22, which were separated by an area that wasn't amplified at 4q13-q21. Through fluorescence in situ hybridization of tumor metaphases, it was found that the Cdk6 and Met sequences that were amplified were in areas that usually stained the same color (HSRs). In three of the tumors, both gene amplifications were found in the same HSRs. In the other two tumors, the same gene amplifications were found in different HSRs. Also, Cdk6 and Met amplifications were always linked to higher gene expression, which suggests that these genes may be the ones that the amplifications were trying to affect. Met and its ligand, the normally quiet hepatocyte growth factor gene (Hgf), were found to be working together in the sixth tumor. Met sequences were also repeated a lot in this tumor, but not Cdk6 sequences. This finding strongly suggests that the tumor has an autocrine signaling circuit. When looked at as a whole, our results suggest that endometrial cancers in the BDII rat may be caused by increased expression of Cdk6 and/or Met.

Keywords: chromosome; Cdk6; HSRs; tumor; hepatocyte.

Introduction

Several pieces of evidence show that neoplastic disease needs a certain sequence of genetic changes, from single nucleotide changes to major chromosomal rearrangements, in a cell lineage (1). DNA amplification is thought to be one of the most important molecular pathways that lead to the growth of tumors. This is because it helps tumor cells turn on more genes whose products help cells grow (2). More and more people are paying attention to gene amplification in human cancers. A bad prognosis is linked to a tumor that grows quickly (3–5). Most of the time, a proto-oncogene has been found to be the likely target of selection when a chromosome region is amplified (4). Some tumors' amplified sequences have a very simple structure, with only the target gene being repeated over and over (6, 7). Amplification usually leads to sequences that are structurally complicated and have been rearranged inside. There are both the target gene and one or more coamplified genes (8, 9). Gene amplification is common in many types of tumors and has important biological and clinical effects on tumor growth. This makes amplified chromosomal regions good places to look for genetics information. Cancer is a complicated disease, and to understand where it comes from and how it spreads, we need to find and describe the genes that are amplified. A similar approach could also lead to the creation of molecular markers that can be used to predict a patient's prognosis and help doctors decide how to treat them. Endometrial carcinoma, which is another name for uterine corpus carcinoma, is the most common cancer in women's reproductive organs and the fourth most common cancer in women around the world (10). 3 But the unique molecular and genetic features of this tumor are still not well understood. The inbred BDII rat strain has a high risk of passing on hormone-dependent EAC4 (11), which means it could be used as a genetic model system for this cancer. For example, a cytogenetic study found a cluster of uterine tumors (mostly EACs) in the F1, F2, and backcross offspring of BDII rats and rats of either of two non-susceptible strains (BN and SPRD). This showed that HSRs, which are signs of gene amplification, were always present in this group. Using CGH, we found that the sequences that were amplified came from a close-by RNO4 region that included bands q12–q22 (12). Similar amplification in the next-door part of RNO4 has been found in some 7, 12-dimethylbenz[a]anthracene-induced rat sarcomas (13–15), which suggests that this part of the chromosome is involved in a big way in many different types of tumors. In this study, we looked at the amount and quality of the amplification of RNO4-derived sequences. Based on what we found, it seems likely that these tumors are caused by independent amplifications in at least two different areas. Each of these places has genes that can cause cancer, and they may work together to cause endometrial cancer.

doi.org/10.31838/hiv22.02.602 Received: 30.04.22, Revised:12.07.22, Accepted: 17.08.22

Materials and Methods

Cells and tissues that have cancer

Endometrial cancer is more likely to happen in females of the inbred rat strain BDII/Han (EAC; Refs. 11 and 16). Most young women under 24 years old go through this change. 90% of virgins go through this change. When BDII/Han rats were bred with rats from two strains that didn't get tumors, BN/Han and SPRD-Cu3/Han, many of the F1, F2, and backcross animals got tumors. Most of these tumors were called embryonal carcinomas (EAC), but pathologists also found a few other uterine and extrauterine tumors (see Ref. 12). For this study, 12 tissue cultures were grown from 11 endometrial tumors (10 of which were EACs and 1 of which was an ESCC). One peritoneal mesothelioma tumor was also used. The DNA was taken out using the standard method of phenol and chloroform.

An Analysis of Cytogenetics and Preparations of Chromosomes Using 2n = 42 cultures of normal rat embryo fibroblasts (for single-color and two-color FISH mapping) and 12 cultures of tumor tissue, chromosome preparations were made (for DNA sequence amplification and cytogenetic analyses). Rat embryo fibroblasts were treated with ICRF-145 [(4,4-(1,2-ethanediyl)bis(2,6-piperazinedione), 25 _M; Funakoshi, Tokyo, Japan] for the last 60 minutes and Colcemid (0.05 _g/ml; Life Technologies, Inc., Grand Island, NY) for the last 20 minutes to make spreads with longer chromosomes, mostly prometaphases (17). Colcemid was used to treat tumor cells in the lab, but not ICRF-145. Mitotic shake-off was used to collect the cells, which were then spun into clusters and resuspended in 0.075 M KCl at room temperature for 30 minutes to let them start the mitotic process again. It was said before that methanol-acetic acid fixation was done (18). After G-banding, cytogenetic analysis was done on the metaphases of the tumor tissue (18). Ten to twenty tumor cells from each tumor were used to make a karyotype. CGH was done again, just as was said before (12).

FISH is used to find out where PAC clones are physically and to look at their DNA sequence. We used pure 200–1200 bp pieces of genes of interest or full-length cDNAs as probes to search a rat PAC library, RPCI-31, for genomic DNA clones (BAC-PAC resources; Roswell Park Cancer Institute, Buffalo, NY). The goal was to get probes that could be used with FISH (for Hgf and Met). Specifics about keeping the PACs separate and keeping an eye on them have been talked about elsewhere (19). PACs were used as probes in both single-color and dual-color FISH, and the procedures almost exactly followed the written protocols (19, 20).

Brings down the South

EcoRI was used to cut up 15 g of genomic DNA from tumor tissue cultures and the normal rat BDII liver used as a control. Using the random priming method, the 200–1200 bp PCR products for the genes and the Hgf and Met cDNAs were each radioactively labeled with [_-32P]dCTP and then hybridized to the filter (21). After washing the filter well, it was put in front of the X-ray film (Hyperfilm-MP; Amersham, Buckinghamshire, United Kingdom). After developing the film, we compared the X-ray hybridization signal intensities between tumor and normal control BDII DNA and used the specialized software Quantity One, ver 4.2.2 to estimate the level of amplification for each gene (Bio-Rad Laboratories, Hercules, CA). 5 Signals from a 146-bp _-actin control probe were used to account for small differences in sample loading. This probe was made using PCR with primers 5 -cacggcattgtaaccaactg- 3 and 5 -ctgggtcatctttcacggt-3_.

RT-PCR

With the help of the RNeasy Mini kit, cytoplasmic RNA from tumor tissue cultures and rat embryonic cells was taken (Qiagen, Valencia, CA). cDNA was made by following established procedures and using the Superscript preamplification system (Life Technologies, Inc.). For each of the 15 genes, 100 ng of tumor cDNA was used as a template for amplification with -actin primers (5 -cacggcattgtaaccaactg-3_ and 5 -ctgggtcatctttcacggt-3_, which made a 146-bp fragment) and -actin primers (5 -ctgggtcatctttcacggt-3_). In addition to the standard PCR parts, 0.4 mM [F]-dUTP R110 from PE Applied Biosystems in Foster City, California, was added to each 10-mL reaction mixture. Denatured at 94 degrees Celsius for three minutes, the first step was followed by 28 cycles of 55 degrees Celsius to 72 degrees Celsius for one minute each. The last step was to keep the temperature at 72 degrees Celsius for 7 minutes. After PCR, a 2-l sample was taken and run through an electrophoresis on TAE-buffered agarose gels. For a semiquantitative analysis, the last 8-liter aliquot was treated with ethanol and put through a PAGE separation in an automated fluorescent DNA sequencer 377. (PE Applied Biosystems). Genescan Analysis Software was used to collect and analyze this data (PE Applied Biosystems).

Rat Met protein was looked at with a Western blot

Most Western blot experiments were done in reducing conditions, using methods that had already been written about (13). In short, proteins were taken out of lab-grown tumor cells by sonicating them. Twenty micrograms of protein were separated on NuPage 4-12% Bis-Tris gels and then electroblotted onto a Novex PVDF membrane (Novex, San Diego, CA) using a Novex Xcell II system (Novex, San Diego, CA). The membranes were blocked for another night after being in a Tris-buffered saline buffer with 10% dry milk (50 mM Tris-HCl, 137 mM NaCl, and 0.1% Tween 20). After that, the blots were probed with a rabbit polyclonal antimouse Met antibody (SP260; Santa Cruz Biotechnology, Santa Cruz, CA) and then

with an antirabbit horseradish peroxidase-linked F(ab)2 fragment (Amersham). After cleaning the membranes, they were put in the same room as a better chemiluminescence detection system (Amersham).

Rat Met Exons 17–19 DNA Sequencing. With primers 5 - ccaccccaatgttctctcac-3_ and 5 - ggtggtgaacttttgcgtct-3_, PCR amplification was done on 100 ng of cDNA from each of the tumor tissue cultures to look for mutations in Met exons 17, 18, and 19. This 382 bp fragment was cleaned using GFX spin columns (Amersham Pharmacia Biotech, Piscataway, NJ) to separate gel bands before it was sequenced using Prism BigDye Terminator chemistry (PE Applied Biosystems). After the sequencing products were fixed in ethanol, they were put through a fluorescent DNA sequencer, model no. 377, which was automatic (PE Applied Biosystems). Sequencing Analysis version 3.3 software was used to look at the DNA sequence (PE Applied Biosystems). Both ways of sequencing the DNA were done with each of the two PCR primers. The standard was made up of cDNA taken from mature rat embryonic cells.

Results

The results of CGH and cytogenetic analyses of uterine tumors in rats are what was expected. From 11 tumors in the uterus of rats (10 EACs and 1 ESCC) from F1, F2, and backcross animals in the two crosses, tissue cultures were made. The cytogenetic analysis of the tissue cultures showed that most of the EAC tumors had between 53 and 69 chromosomes (2n). However, RUT2 and RUT7 were almost diploid, and NUT51 and NUT82 were also in the near-tetraploid range. As a comparison, a tissue culture of RUT29, another type of mesothelioma, showed that it was hyperdiploid (2n = 44). Gene amplification was shown by HSRs of different sizes, which were present in 11 of the 12 tumor cultures (Fig. 1). CGH analysis could find each cultured tumor's amplified sequences and a rough idea of where they came from (Table 2; the criterion used was a green:red fluorescence ratio of 2.5). The data set shows that at least eight different parts of the chromosome were amplified in this material, and some of them were amplified more than once. These areas were likely found in all tumor cultures with HSRs. Most of the time, amplifying happened in the area called RNO4q12-q22 (Fig. 2). This matched what CGH found in the primary tumors that were used to make the tissue cultures. In fact, 11 (58%) of 19 primary malignant EACs and 12 (55%) of 22 tumors, including three more tumors called uterine sarcoma, uterine carcinoma, and peritoneal mesothelioma, showed moderate- to high-level gain of sequences from RNO4 (12). Also, the fluorescence ratio curves of 10 (45%) of the 22 primary tumors showed high-level copy number increases that were only in RNO4q12-q22. In other words, this data pointed to gene amplification, which is the process of making five times as many copies of a gene as usual (Ref. 2; Fig. 2).

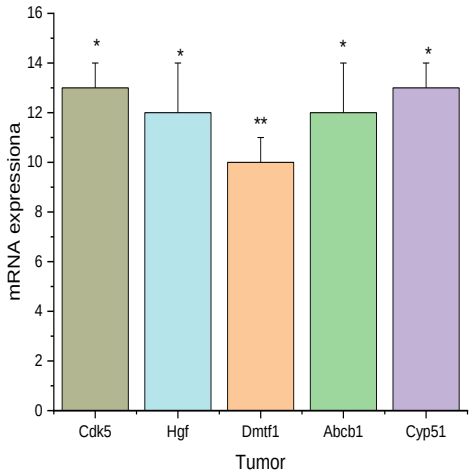

Fig. 1. mRNA and Met protein expression in the rat tumor tissue cultures studied

Putting together a physical and topographical map of the area around RNO4. A complete molecular cytogenetic characterization of the area was needed to find possible tumor-related loci and define the amplified RNO4 segment. With the mapping data for HSA7q21 and 7q31-36, we can compare.

Table 1. mRNA expressiona

TUMER	*Asns*	*Cav1*	*Met*	*Wnt2*	*Cftr*	*Smoh*	*Braf*	*Arhgef5*	Met protein
R1	12.544	8.987	2.099	45.678	5.996	77.246	34.001	12.34	102.001
R2	12.654	8.660	2.099	45.555	5097	77.988	35.002	12.56	103.001
R3	13.554	7.567	2.076	46.876	5.076	76.886	34.001	11.64	120.002
R4	13.426	8.345	1.024	45.444	3.086	76.456	35.011	23.56	103.001
R5	12.863	7.768	2.024	46.333	4.087	75.555	36.022	34.98	102.001
R6	12.077	7.555	2.077	46.226	3.076	76.333	33.001	12.58	103.003
R7	12.876	8.988	2.099	46.777	3.087	75.344	32.011	12.55	103.001
R8	11.455	7.233	2.066	46.098	5.765	75.098	33.003	11.44	103.002
R9	11.544	8.887	2.044	45.567	4.089	75.766	34.011	13.76	102.001
R10	12.456	7.222	1.066	45.222	5.676	75.098	35.001	12.67	102.002
R11	12.123	7.888	2.022	46.231	2.546	76.098	34.002	12.45	102.001
R12	12.779	8.876	2.011	45.123	4.345	76.545	34.001	12.34	102.002

*For the RT-PCR and Western blot tests, total RNA and proteins from the 12 rat tumor tissue cultures were used.

We found putative genes on mouse chromosomes 5 (0-12 cM) and 6 for further mapping and amplification studies (0–15 cM). We used dual-color FISH on rat metaphase and prometaphase chromosomes, as well as on interphase chromatin in resting cell nuclei, to figure out the sublocalization, relative order, and distances between 15 loci. More about this can be found elsewhere (19). All of the chosen genes were found to be linked to RNO4, so using the cytogenetic band, the following map was madeTaking into account the same human genes on HSA7q, the order of the genes and the distances between loci are very similar (19).Changes to the number of copies of a gene near the RNO4 region In the 12 tumor tissue cultures, the number of copies of each of the 15 genes spread across bands q11-q23 of RNO4 were counted. This was done to map the amplified area on RNO4 and find the smallest region or regions of common amplification.

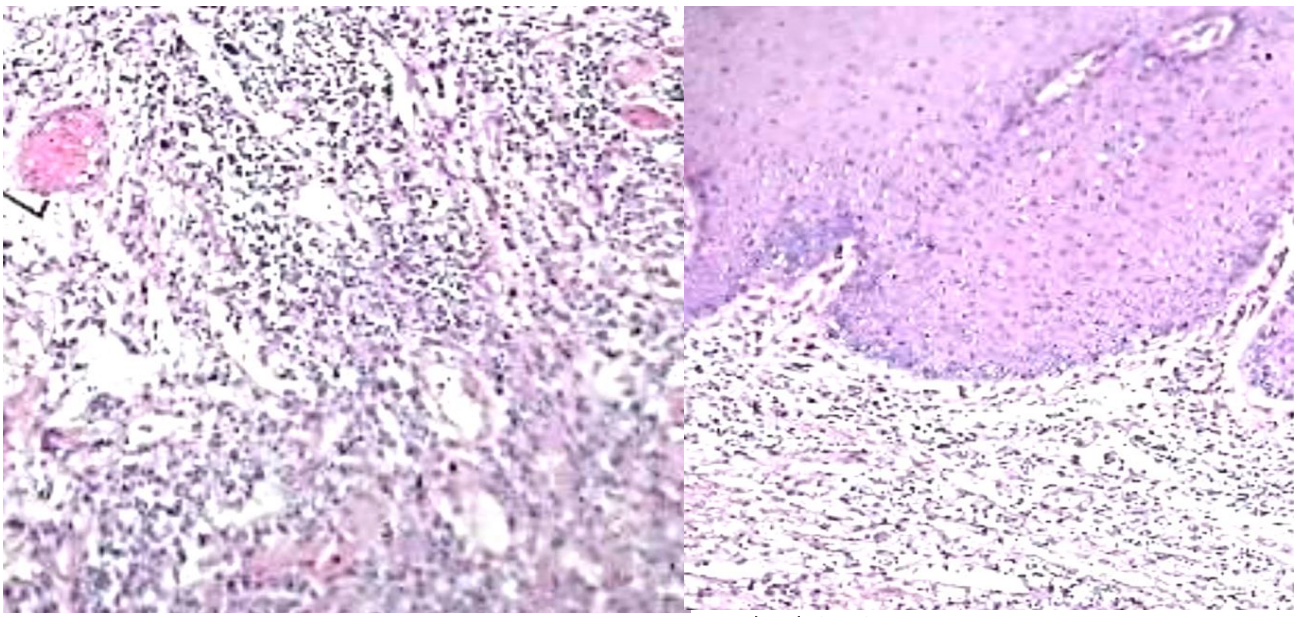

Figure 2: Measuring tumor depth (40X)

They looked at the Southern blot

Each PCR-made probe was placed on a filter, along with an equal amount of digested DNA from each tumor sample and the normal control sample (liver DNA from a BDII animal). Figure 3 shows how things turned out. After hybridization, the bands were looked at with a densitometer. The level of amplification for each probe was guessed by comparing the signal strength of each tumor sample to that of the other samples.the sample from the normative control group. To tell the difference between gene amplification, low-level RNO4 copy number increases (like trisomy), and hyper-diploidy, only increases of _2.5-fold, which is equal to _5 copies/DCSE, were scored as significant. Some genes had an unusually high number of copies (amplification).There were 15 genes in six of the 12 tumor cultures that were looked at (RUT5, 7, 13, 29, NUT51, and 82; Fig. 4). The Southern blot results for five of these tumors agreed with the CGH results, which showed fluorescence ratios of 2.5 in the RNO4q12-q22 area (RUT7, 13, 29, NUT51, and 82). (Table 2). The CGH analysis of the sixth tumor, RUT5, found no major changes in the number of copies of DNA in RNO4. But CGH has a low resolution, so an increase in the number of DNA copies in a very small area (_2-4 Mb) might not be seen (22). When CGH was used to measure the average fluorescence ratio for RUT13, two peaks were found in the RNO4q12-22 region. Because of this, the near-green signal-to-noise ratio and far-green signal-to-noise ratio in each metaphase of the composite (FITC/rhodamine) CGH images of RUT13 were different. the piece of RNO4 (Fig. 5A). Metaphases of the tumors RUT29, NUT51, and 82 were also looked at, and sometimes they had the same pattern of hybridization. The results of this study showed that there might be overlap or closeness between two amplicons in these four tumors. All told, there were Southern blotting analysis proves that bands 4q12-q13 and 4q21-q22 were the source of two different amplified regions. Figure 4 shows that all six tumors had more copies of the more distant region (4q21-q22) than of the more nearby region (4q12-q13).

Besides RUT7

Between the two amplified regions was a region with the Asns and Tac1 genes. These two genes are located in the 4q13-q21 border region. So, in RUT29, the copy numbers of Asns (Fig. 3) and Tac1 were within the normal range (_2 copies/DCSE), but in RUT13 (_3 copies/DCSE) and NUT82, they were a little bit higher (3–4).

copies/DCSE). The analysis showed that there were fewer copies of the RUT5 and NUT51 genes (_1 copy/DCSE). It was clear that the two boosted regions had different amplicons. Instead, their lengths and levels of amplification were different for each type of tumor. In the RNO4q12-q13 region, among the gene probes with significantly higher copy numbers, Cdk6 amplification was the highest.

The levels of amplification ranged from ten times in RUT29 to fifty times in NUT82 (Fig. 1).Amplification of cyclin p51 (Cyp51) was also found in four of the five tumors that were studied. The amount of amplification ranged from 5 times in RUT29 to 30 times in RUT13 and NUT82, but it was much lower than in Cdk6. Abcb1 was also found in NUT82, but it was there less often (_5 copies/DCSE). And in RUT29 (_6 copies/DCSE) for both Abcb1 and Dmtf1. In all six tumors with distal region amplicons, the genes Cav1 and Met were always found together (Figure 3). In both cases, the amount of DNA was increased by between 6-7 fold (in RUT7) and _75 fold (in RUT13).
Data showed that in most tumors, the number of copies of Cav1 was slightly lower than that of Met, even though it was harder to figure out the exact number when there was high-level amplification. In RUT29, where Cav1 was multiplied 33-fold and Met was multiplied 50-fold, the difference between the number of copies of each gene was the biggest. Wnt2 amplification, along with that of Cav1 and Met, was found in five tumors (RUT7, 13, 29, NUT51, and 82). It was thought that Wnt2 would be copied anywhere from four to thirty times as often in RUT7 and RUT13. Also, Cftr was amplified in four tumors (RUT7, 13, NUT51, and 82), and its estimated copy numbers were very similar to those of Wnt2. Three out of the four tumors with amplified Cftr sequences had high levels of Smoh.
like RUT7 (_6 copies/DCSE) and RUT13 (_5 copies/DCSE). We were surprised to find low-level amplification of Smoh (_6 copies/DCSE) in RUT29, a tumor with a normal number of Cftr copies (2 copies/DCSE). Even though these results are not conclusive, they suggest that there may be three different amplicons in this tumor. This particular tumor sample had gene probes with copy numbers lower than the expected 2 copies/double-stranded eukaryotic ribosomal RNA (DCSE) (Fig. 4). People used to think that for every digital content creation system, there were 1.5 copies (DCSE).
The ability of genes to surround amplification hotspots was tested, and they were ranked based on this. In RUT5, there were fewer copies of the genes Wnt2, Cftr, and Smoh. In RUT13, there were fewer copies of the genes Hgf, Dmtf1, and Abcb1. In NUT51, there were less copies of Tac1, Asns, and Smoh. Cdk6, Tac1, and Asns were found to have the fewest copies (_0.5 copies/DCSE) among the genes in RUT7. As was already said, this tumor had a single, weakly amplified amplicon that matched the distal region.
The evidence suggests that the bands 4q13-q21 were taken out of RUT7 to make room for the amplified bands 4q21-q22.

Placement of amplified sequences on specific chromosomes

Isolated PAC clones were used as probes in FISH on chromosome slides made from six tumor tissue cultures with RNO4 amplification. This showed where the amplified loci were and how different the cells were in each tumor. All of the tumors had hybridization signals at the "native" site of the gene in the RNO4q12-q22 region, which showed that the chromosomes were still whole. Using the Southern blotting method, it was found that all six tumors had signs of amplified gene expression. In the nuclei of cells that were not doing anything, these gene probes were found to be strongly hybridized in groups. Most of the HSRs on the metaphases with extra signals were on chromosomes far from where the RNO4 genes were normally found. The HSR signals spread out in different ways. Some of them crowded together in tight groups, while others spread out "chromosome
paint-like "letting longer HSRs have structures that are less packed together. The estimated copy numbers from the Southern blot matched up well with the signal counts, but the exact number of signals could not be found in cases of high-level amplification. It was also found that the kinds of cells in each tumor were different.
was big in terms of the number and order of probe signals, but not so big in terms of the size and number of HSRs. Five tumors, each with two amplicons, had their metaphase spreads cohybridized with PACs for Cdk6 (4q13) and Met (4q21.2) to find out where the amplified sequences were in each of the two amplified subregions.
RNO4. Each of the two probes in RUT13 and RUT29 was linked to a different HSR. Southern blotting showed that RUT13 had a lot of Cdk6 and Met sequences. Notably, the Met signals came more often than the Cdk6 signals. Most of the metastases from this tumor have the Cdk6 gene.probe set off a pattern of hybridization on a single HSR that was consistent and close together. Mutated Met sequences were always found in two HSRs in the same tumor. These HSRs were different from the HSRs that had Cdk6 sequences that were amplified. The Met signals on these HSRs did not have the same regular pattern as the Cdk6 signals.
making a stair-step pattern of areas with a lot of hybridization and areas with little hybridization (Fig. 5B). Also, chromosomal sublocalization of signals showed that, unlike Cdk6 signals, Met signals were only in two small areas of the interphase nuclei.
was kept as a single unit (Fig. 5C). We found that RUT29 had 20 copies of Cdk6 on a single HSR and highly amplified Met on two HSRs by counting signals. The Met signals on the HSRs were spread out like rungs on a ladder, just like in RUT13. In RUT13 and RUT29, the Cdk6 and Met probes didn't interact with the same HSRs, but they did in RUT5, NUT51, NUT82. Both probes matched up with two HSRs in RUT5 at the same time. The larger one was on a large subtelocentric chromosome, and the smaller one was at the end of RNO4. Also, it was seen.The number of Cdk6 copies in a group of RUT5 cells stayed the same, but the number of Met copies changed. Based on the measurements of signal intensity, each cell had between 50 and 60 copies of Cdk6, while 90% of the cells had between 15 and 20

copies of Met and 10% showed high-level Met amplification. In the last few cells of the larger of the two HSRs, Met signals formed very tight, ladder-like structures. On the other hand, Cdk6 signals were spread out evenly across both RUT5 HSRs, but there weren't many of them. Many hybridization signals from the Cdk6 and Met probes in NUT82 were spread out along two long HSRs. Most of the time, the Met signals were spread out evenly, but the Cdk6 signals on the HSRs were spread out in a way that looked like a ladder. From what was known about metaphases, it was expected that the Cdk6 and Met signals would be close to each other in interphase nuclei (Fig. 5D). Even NUT51, which also had two long HSRs, did the same thing when it came to hybridization. The signal counts, on the other hand, showed that there were fewer copies of Cdk6.

Based on what NUT82 says

messenger RNA Expression. DNA replication in tumors usually leads to more expression of the duplicated gene (3). Because of this, RT-PCR was used to look at the amount of mRNA in cultures of tumor tissue. We co-amplified each gene with _-actin by using an equal amount of cDNA from each tumor as a template. This is what we learned The amount of _-actin in tumors didn't vary much. We put each tumor's expression into a category based on how many fragments of the tested gene and _-actin were in the tumor. At least one of the 12 tumors had to have a small amount of tumor tissue for mRNA to be found for all 15 genes (Table 3). Only about 25% of tumors had three copies of the same gene (Hgf, Abcb1, and Tac1). Very low amounts of Tac1 mRNA were found in RUT25, while RUT7 and RUT25 had moderate and low amounts of Hgf mRNA, respectively. in RUT30, etc (Fig. 6). Five other genes (Cdk5, Dmtf1, Asns, Smoh, and Braf) were also found to have low levels of expression in most tumors. For example, Dmtf1 was barely expressed in tumors, except for RUT3, where it was even less so. Simple language that is used over and over again come to help Smoh. Asns expression was also low in most tumors. It was missing in RUT5, 7, 13, 29, and 30, and it was very low in the rest. High levels of the gene Arhgef5 were found in all tumors. The concentrations of RUT7, 12, 25, 29, and 30 were pretty high, but the concentrations of the other genes were either very low or couldn't be found. In the Southern blot analysis, the only genes that showed strong expression were the ones with significantly more copies. about the same number of copies of the Cdk6 gene were made as mRNA transcripts. The four tumors that had the most copies of the Cdk6 gene showed the most expression (RUT5, 13, NUT51, and 82; Fig. 6). Also, RUT13 had a 30-fold increase in Cyp51, making it the tumor with the most Cyp51 expression. RUT5 and 7 were the tumors with the least Cyp51 expression. In the last two tumors, there were less copies of Cyp51 (_1.5 copies/DCSE). For the Met gene, which is in the distal amplicon, there was a strong link between the amount of mRNA and the number of copies. Southern blotting showed that the four tumors with the most copies of the Met gene also had the most expression of the Met gene.
showing how the Met protein works. Met, which is an oncogene, was looked at along with the Met mRNA levels in the tumor tissue cultures to see how it was expressed.
With an antimouse Met antibody, a Western blot was done on both the tumors with Met amplification and the ones without it. Except for RUT3, all of the tumors that weren't amplified had very little or no Met protein. It was already known that this tumor had many cells with three working copies of the RNO4 gene (12). In the six tumors with Met amplification, the Met protein, Met mRNA, and Met copy number were all found to be in good agreement. RUT13, 29, NUT51, and 82, but not RUT7 and RUT5.
The study of changes in the Met gene of rats. Point mutations in the tyrosine kinase domain turn on the MET proto-oncogene in both familial and sporadic papillary renal carcinoma (23), as well as in lymph node metastases of head and neck squamous cell carcinomas (24).
Changes in the tyrosine kinase domain in exons 17–19 were looked for in DNA from 12 cultured tumors to see if Met was turned on by a mutation in this set of rat tumors. When the obtained DNA sequences were compared with rat Met sequences in the GenBank DNA sequence database, all of the tumors had a C3T change at the third position of codon 1172 in exon 17. (AAT3AAC). Since both AAT and AAC code for the amino acid asparagine, it was first thought that this mutation was a "silent polymorphism," which means that it didn't change the structure of the protein. This sequence change was interesting because it seemed to be the same for all three strains that were tested in this study.

Discussion

The EAC-prone BDII rat was used to study how genes affect the growth of EAC in humans. With the help of cytogenetics and CGH, uterine tumors (mostly EACs) that grew on their own were studied in the offspring of BDII rats and two other rat breeds.
pathogen-resistant strains (12). (12). Most of the time, amplification happened near the beginning of RNO4. This was true of both the original tumors and the tissue cultures that were made from them. We made a panel of large-insert genomic PAC clones for positional localization of candidate oncogenes using PCR-generated probe fragments that represent genes thought to be in the area (19). We used FISH and Southern blotting to find out where in the genome RNO4 was amplified in tissue cultures from 10 EACs.
There was one ESCC and two cases of peritoneal mesothelioma. In six of the tumors, we found RNO4-derived amplification units that were different from the others, and in two of the tumors, we found two different areas of shared amplification.
Natural selection is affecting at least two target genes (4q13 and 4q21.1-q21.2). Coamplification of

two different syntenic regions on HSA17q is common in human breast cancers (25, 26). Most of the time, the gene Cdk6 at 4q13 was amplified, but up to four of the tested genes (Dmtf1, Abcb1, Cyp51, and Cdk6) were also part of the closest amplified segment. Cdk6 was by far the most important of the genes that were amplified in tumor amplicons. When Cdk6 copy number was high, it was linked to with more Cdk6 messenger RNA, which happens when a gene is duplicated and turned on too much. CDK6's human homologue, CDKN2A, maps to 7q21-q22. CDK6 is thought to be an important part of controlling the cell cycle because it links growth factor stimulation to cell divisio beginning of the cell cycle (27). (27). Harbour et al. (28) have shown that CDK6 is one of the CDKs that phosphorylates the RB1 protein. This stops the protein from stopping growth in the G1 stage and makes it easier for the cell to move into the S phase. Even though CDK6 has some traits that should, in theory,

Changes in CDK6 that cause cancer in humans are rarely talked about. Even when CDK6 is not amplified, some tumors still have too much CDK6. Overexpression of CDK6 was seen in T-cell lymphoblastic lymphoma/leukemia (29), as well as in splenic marginal zone lymphomas.

Carriers of a certain HSA2-7 translocation, where the breakpoint in HSA7 was upstream of the CDK6 transcription start site (30). We found that CDK6 overexpression was only seen in high-grade glioblastomas (31) and not in less dangerous ones. Increasing the amount of CDK6 expression seems to be good.

would probably cause the growth of these tumors to speed up. CDK6 overexpression and CDK6 amplification have only been linked to human gliomas (32). (32). The authors thought that the amplification only affected CDK6 because they couldn't find any evidence that other genes on HSA7 were also amplified.

Expressed sequence tags near the CDK6 locus, the EGFR gene at 7p11-p12, and the MET gene at 7q31. In the second region, the amplified sequences included up to five of the tested genes (Cav1, Met, Wnt2, Cftr, and Smoh), and the center of the amplification was near Cav1 and Met at 4q21.1–21.2. In all six tumors with RNO4 amplification, the expression of both genes was very high. Instead, the number of copies needed to meet the goal stayed the same.

Usually a little bit higher than Cav1's. Also, the link between amplification and expression was stronger in Met than it was in Cav1. When Met amplification happened, both the amount of Met mRNA and the amount of Met protein went up. Met was 20 times stronger in RUT5, but the levels of its mRNA and protein were the same as RNO4-trisomic RUT3. RUT5 was an ESCC, but none of the other tumors were (except RUT29)

were EACs. Both CAV1 and MET have been linked to cancer because of their functions and their shared location at 7q31 in humans. Caveolin is a scaffolding protein that is made from the CAV1 gene. It helps to keep certain lipids and signaling molecules that have been changed by lipids together and in the right place in the membrane.

called caveolae (33). Caveolin stops these signaling molecules from doing their jobs, which supports the idea that CAV1 might be a gene that stops cancer from growing (reviewed in Ref. 34). (35). The MET proto-oncogene was first found as a transforming gene that was turned on by translocation in a human osteosarcoma cell line that had been chemically changed (36). HGF/SF is the ligand for the transmembrane growth factor receptor tyrosine kinase that is made by MET (37). There are many different kinds of cells, but epithelial cells have the most MET receptor tyrosine kinase. Based on the fact that mesenchymal cells are the main source of this factor, it is thought that HGF/SF has paracrine effects on MET-expressing cells (38). Signals sent by HGF/SF-MET interactions may have an effect on proliferation.

and movement of endometrial epithelial cells among other types of epithelium (39, 40). In vitro studies have shown that MET-positive endometrial carcinoma cell lines can spread faster when HGF/SF is present (41).

So, based on what we know about CAV1 and MET and how they work, we can say that MET is a better candidate target for gene amplification. Several news stories have Researchers have found proof that MET encourages the growth of tumors. Multiple cancers in people have been found to have more MET transcripts, which suggests that the receptor may have more than one job. Even though MET overexpression has been found in a number of cancers, including those of the stomach (5 cases), ovaries (42 cases), breasts (43 cases), thyroid (44 cases), and bowel (45 cases), there isn't much evidence linking these cancers.Mucosal tumors (45), GI stromal tumors (46), and uterine sarcomas (47). (47). (47). In humans, amplification of the MET gene has only been observed in gliomas and gastric cancers (48, 49). (48, 49). This is a new study because it is the first one to show that Met overexpression and amplification happen together in endometrial carcinomas.

There have been reports of point mutations in human MET. These mutations are what turn it on in some tumors (23, 24). All of the tests for Met point mutations turned out to be negative. This is very unlikely, at least in tumors that are getting bigger. that the DNA sequence of a gene rarely changes after being copied.

Instead, it seems that the wild-type gene that hasn't been changed is what makes tumors grow (50).

Cancer of the uterine lining.The main benefit of using an animal model for a complex human disease like endometrial cancer is that the genetic and environmental differences between humans and animals may be greatly reduced. You might think that there aren't many genetic changes in tumors because it's clear that the BDII strain makes people

more likely to get EAC tumors on their own and because there aren't many changes to the gene pool or the environment. Cytogenetics and/or CGH could find a wide range of big changes to the chromosomes in these tumors (12). Gene amplification was found in a total of 15 places.

There were a total of eight different chromosomes in 11 different tumor cell lines (Table 2). This shows that when cancer starts to grow, many different changes to genes can lead to the same result, even in a system with less variation like the one studied here. It is a huge challenge to find and describe all of these possible paths. CGH analysis of our data showed an interesting change: the proximal region of RNO4 was amplified in five tumors. After a more detailed analysis had been done, RUT5 was added to the mix.using probes that are specific to many genes. So, it looks like chromosomal amplifications in this area are a big reason why cancer is more likely to happen in this model. The results of the pathology showed that the six tumors with proximal RNO4 amplification were from three different types of tumors.analysis. But the BDII strain makes four of them more likely to get an EAC tumor. These are RUT7, 13, NUT51, and 82. Only the farthest RUT7 segment showed amplification, and as we've already talked about, the presence of Hgf expression in this tumor points to the possibility of a growth-stimulating mechanism involving an autocrine loop, which may contribute to the fact that the tumor is malignant. Cdk6 and Met were amplified and overexpressed in three epithelial adenocarcinomas, one epithelial squamous cell carcinoma, and one peritoneal mesothelioma.

shows that they may work together in a way that helps the transition to cancer, but the details of how they work together are still not clear.

Conclusion

It is impossible to say for sure that the amplicons don't have any more cancer-related genes that could affect the growth of tumors. But Cdk6 and Met are likely to play important roles in the growth or development of the rat tumors that were studied because they are known to help the cell cycle progress and help cells grow and move, respectively. It would be interesting to find out if the same pathways lead to the growth of endometrial cancer and other cancers in humans.

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