Breast cancer is a heterogeneous tumor entity resulting from several molecular progression pathways (1). Tumors expressing basal epithelium cytokeratins (CK5/14/17) constitute a subgroup distinct from basal cytokeratin-negative luminal epithelium-like tumors. Basal cytokeratin-positive tumors are characterized by hormone receptor negativity, a high tumor grade, and a high proliferation activity (2–9). Gene expression microarray studies have also distinguished the “basal-like” tumors from the luminal, ERBB2-overexpressing, and normal breast-like tumors, and have associated the basal-like group with adverse patient survival (1, 10–12). By immunohistochemistry, the expression of CK5/14/17 can be detected in ~10% of breast tumors (2, 5, 7, 13). The overwhelming majority of breast cancers are characterized by the expression of CK8/18 only, the predominant cytokeratins of the glandular epithelium lining the lumen of the breast ducts (2, 5, 7, 14). Therefore, in most immunohistochemical studies, the classification of breast cancers into “basal phenotype” and “luminal phenotype” subgroups refers to the presence or absence of CK5/14/17 in the tumor cells, respectively (2, 4, 6, 7), because CK8/18 can be detected in all sporadic CK5/14-positive breast tumors (2). Immunohistochemically, the CK5/14-positive but CK8/18-negative phenotype is typical for hereditary breast tumors from BRCA1 germ line mutation carriers (2).

Besides negative estrogen receptor status, several biological markers have been associated with breast cancers expressing basal cytokeratins. Vimentin, c-kit (CD117), epidermal growth factor receptor (EGFR) and p53 are more frequently overexpressed in basal than in luminal phenotype breast cancers.
(5, 12, 15–18). The Bcl-2 expression, on the contrary, is more likely to be low or absent in tumors expressing basal cytokeratins (5). Based on the gene expression microarray studies, breast cancers with HER-2 (ERBB2) amplification have exclusively been grouped into an entity that is distinct from the basal-like tumors (1, 10–12).

Basal cytokeratin–positive breast cancer has been defined variably as showing “any cytoplasmic staining” (5, 7, 12) or positivity in at least 5% (3), 10% (13), or 20% (2) of the tumor cells. Tumors expressing basal cytokeratins are thought to originate from CK5-positive epithelial progenitor cells of the breast (14, 19, 20). In the normal breast, these cells are capable of differentiating into luminal epithelium (CK8/18+) and myoepithelium (expressing smooth muscle actin) lineages via intermediate phases (14, 19, 20). In tumors, the cytokeratin profile is thought to remain stable after malignant transformation (21), thus reflecting the differentiation stage of the cancer precursor cell. Heterogeneous basal cytokeratin expression could reflect pathogenetic significance. In an experimental model system, activation of different oncogenic pathways lead to tumors that either expressed basal cytokeratins heterogeneously or express only luminal cytokeratins (22).

In this study, we determined the basal cytokeratin expression pattern in breast tumors using image analysis. The results were correlated with the biological and clinicopathologic tumor characteristics and patient survival.

Materials and Methods

Tumor samples. We studied a population-based cohort of 506 primary invasive breast cancers by using a cocktail of three immunohistochemical basal cell markers (CK5, CK14, and p63) as described previously (2). Of the 506 breast cancers, 53 tumors in which at least 5% of the tumor cells were positive for CK5/14 were studied in detail. For comparisons between CK5/14-positive and CK5/14-negative groups, we selected 45 consecutive CK5/14-negative invasive ductal breast cancers and an additional 22 CK5/14-negative and estrogen receptor negative tumors (16 of these were grade 3) from the entire patient cohort. All except two of the basal cytokeratin–expressing tumors were invasive ductal breast cancers. One CK5/14-positive tumor was diagnosed as metaplastic carcinoma and the other as medullary carcinoma. To study the persistence of CK5/14 heterogeneity, we studied four pairs of CK5/14-positive primary tumors and their metastatic lesions. The tumors and clinicopathologic data were collected from the archives of the Department of Pathology at Seinäjoki Central Hospital, Seinäjoki, Finland (with permission from the ethical committee of Seinäjoki Central Hospital).

To evaluate the prognostic effect of the intratumoral heterogeneity of CK5/14, we studied a separate cohort of 382 tumors from a randomized adjuvant chemotherapy trial of high-risk breast cancer patients (Scandinavian Breast Group 9401 trial; refs. 23, 24). In brief, this cohort comprised high-risk breast cancer patients with eight or more positive lymph nodes, or five or more involved lymph nodes and negative hormone receptor status, and either negative anaplasia grades 2 and 3 or a high 5 phase fraction. The patients were adjuvantly treated with either nine courses of dose-escalated, or three to four courses of standard 5-fluorouracil, epirubicin, and cyclophosphamide followed by high-dose cyclophosphamide, thiopeta, and carboplatin supported by autologous bone marrow support. All tumor samples in this study were routinely fixed with formalin, embedded in paraffin and sections of 3 to 5 μm thickness were obtained.

Immunohistochemistry. All cytokeratin immunostainings were done on tissue sections obtained from the original tumor blocks in order to allow examination of the intratumoral heterogeneity. Analysis of HER-2 (by chromogenic in situ hybridization), EGFR (immunohistochemistry and chromogenic in situ hybridization), vimentin, c-kit, p53, Bcl-2, and Ki-67 was done either on original tumor blocks or on tissue microarrays. A survival study was conducted on a cohort of patients from the Scandinavian Breast Group 9401 trial and the material from the original primary tumor sections (24).

The breast cancers expressing basal cytokeratins were identified by using an antibody cocktail CK5/CK14/p63 (NM26, 1:400; Novocastra, Newcastle upon Tyne, United Kingdom); LL002, 1:400 (Novocastra); 4A4+Y4A3, 1:1,500 (Neomarkers, Fremont, CA) as described earlier (2). In brief, the slides were deparaffinized, rehydrated, and subsequently pretreated in 0.05 mol/L of Tris-HCl with 0.001 mol/L EDTA (pH 9.0), in an autoclave at 103°C for 5 minutes. An antihorse radish peroxidase polymer kit (PowerVision+ kit; Immunovision Technologies Co., Brisbane, CA) was used as a detection method with 3,3′-diaminobenzidine as a chromogen.

The proliferative activity was studied using a monoclonal Ki-67 antibody (MIB1, 1:1,000; DakoCytomation, Glostrup, Denmark). A sequential two-color immunostaining was used to characterize the proliferative activity of CK5/14-positive and CK5/14-negative tumor cells among 25 tumors that were heterogeneously positive for CK5/14. The slides were first immunostained with Ki-67 (as described above) by using 3,3′-diaminobenzidine as a chromogen (brown reaction product) and subsequently with the antibody cocktail CK5/CK14 by using AEC (red reaction product). The pretreatment was conducted prior to the first immunostaining. Both antibodies were detected by using the PowerVision+. Hematoxylin was used as a counterstain.

The tumor sections were also immunostained for CK17 (E3, 1:50; Labvision, Fremont, CA), CK8/18 (5D3, 1:400; Novocastra), EGFR (EGFR.113, 1:100; Novocastra), vimentin (3B4, 1:1,000; DakoCytomation), c-kit (polyclonal, 1:200; DakoCytomation), Bcl-2 (124, 1:700; DakoCytomation), and p53 (DO-7, 1:500; Novocastra). The pretreatment and detection methods were the same as described above. To prevent overstaining, p53 was detected by a less sensitive avidin-biotin based detection method Vectastain Universal ABC kit (Vector Laboratories, Burlingame, CA). The amplification of the HER-2 and EGFR oncoproteins was studied using chromogenic in situ hybridization as described earlier (25, 26).

Slide scoring. The percentage of CK5/14-positive malignant epithelial cells was defined using an Olympus BX61 microscope and AnalySIS image analysis software (Soft Imaging System GmbH, Münster, Germany). At least 100 tumor cells were counted from two to five visually selected fields. The Ki-67 labeling index was defined using the same method. For the substudy of survival, CK5/14 staining was classified as negative (<5%), heterogeneously positive (5-69%), or uniformly positive (≥70%) of immunopositive tumor cells. Vimentin, c-kit, Bcl-2, p53, and p63 (nuclear staining with CK5/CK14/p63 antibody cocktail) were regarded as positive when ≥20% of the tumor cells showed positive staining. CK17 and CK8/18 were classified as negative (<5%), heterogeneously positive (5-69%), or uniformly positive (≥70%) of immunopositive tumor cells. EGFR immunohistochemistry was scored in a four-step scale (−, +, ++, and +++), and + and +++ scores were regarded as overexpression.

HER-2 and EGFR were considered amplified when chromogenic in situ hybridization revealed six or more gene copies per cell in at least 10% of the tumor cells.

Statistics. Fisher’s exact test and χ2 test were used to test the significance of the cross-tabulated data [using GraphPad Instat (GraphPad Software, San Diego, CA) and MedCalc (MedCalc Software, Mariakerke, Belgium) statistical software]. Survival analyses were calculated using Kaplan-Meier life table curves and the log-rank test. Relapse-free survival was calculated from the primary diagnosis to the first reported breast cancer–specific recurrence excluding contralateral breast cancer. All reported P values are two-sided.
Results

**Basal and basoluminal breast cancers.** We screened 506 invasive breast tumors by setting the cutoff at 5% of CK5/14-positive tumor cells, which according to our experience, is the lowest fraction that can be defined reproducibly in an immunohistochemical staining. The fraction of CK5/14-positive tumor cells was defined on all resulting 53 CK5/14-positive tumors by image analysis. The distribution of the tumors according to the proportion of CK5/14-positive cells is shown in Fig. 1A. Based on the two-peak distribution of the CK5/14 positivity, we classified the tumors expressing CK5/14 into two subtypes by setting an arbitrary cutoff at 70% of the CK5/14-positive tumor cells. Approximately half of all CK5/14-positive tumors (58%) microscopically showed a distinct heterogeneous immunostaining (median, 32% of positively stained cells) and were called “basoluminal.” On immunostaining, basoluminal tumors often showed a focal checkerboard pattern with CK5/14-negative and CK5/14-positive tumor cells located next to each other (Fig. 1B). The remaining tumors (42%) stained uniformly or almost uniformly with CK5/14 (median, 94% of positively stained cells) and were called “basal” (Fig. 1B).

To further validate the classification based on intratumoral heterogeneity of basal cytokeratin expression, we stained the same 53 tumors with an additional basal CK17 antibody. The CK17 staining type (negative, heterogeneous, and uniform) correlated strongly with the CK5/14 staining type (P < 0.0001; Table 1). To determine whether the CK5/14-positive tumor staining types, basoluminal and basal, remain stable during tumor progression, we analyzed four pairs of CK5/14 expressing primary tumors and their metachronous metastases. All metastases showed the same CK5/14 staining type as was observed in the primary tumor. The only basal tumor metastasized to the chest wall and the three basoluminal tumors metastasized to brain (Fig. 1C), subcutis, and ovary.

**Biological and clinicopathologic characteristics of CK5/14-positive tumor subtypes.** In comparison with the luminal (CK5/14-negative) control tumors, all CK5/14-positive tumors (with at least 5% of positive tumor cells) were strongly associated with a high tumor grade (P = 0.0002), negative hormone receptor (P < 0.0001) and Bcl-2 status (P < 0.0001), and overexpression of EGFR (P < 0.0001), vimentin (P < 0.0001), c-kit (P < 0.0001), and p53 (P = 0.0002; Table 1).

In a similar comparison between the CK5/14-positive tumor subtypes, we observed that both basoluminal and basal tumors were predominantly hormone receptor negative and of a high tumor grade (84% versus 95% estrogen receptor negative, respectively; 74% versus 82% grade 3, respectively). Basoluminal

![Fig. 1. A. distribution of breast tumors according to the proportion of tumor cells staining positively for CK5/14. The classification into heterogeneously positive basoluminal and uniformly positive basal subtypes is shown (5-69% or ≥70% of CK5/14-positive tumor cells, respectively). B. examples of immunohistochemical stainings of CK5/14 of basal and basoluminal subtype breast cancers (original magnification, ×200). C. examples of immunohistochemical stainings of CK5/14 of a primary basoluminal breast cancer and its subsequent distant metastasis (original magnification, ×200).](http://www.aacrjournals.org/ClinCancerRes/article-pdf/12/14/4187/4230367/4187.pdf)
Table 1. Comparison of clinicopathologic features in CK5/14-negative and CK5/14-positive breast tumors and basal cytokeratin – positive subtypes (basal and basoluminal)

<table>
<thead>
<tr>
<th>Clinicopathologic characteristic</th>
<th>CK5/14-negative tumors, % (n = 45)</th>
<th>CK5/14-positive tumors, % (n = 53)*</th>
<th>P</th>
<th>Basoluminal tumors, % (n = 31)</th>
<th>Basal tumors, % (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (&lt;50 y)</td>
<td>16</td>
<td>25</td>
<td>0.32</td>
<td>23</td>
<td>27</td>
<td>0.75</td>
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<tr>
<td>Tumor size (≥2 cm)</td>
<td>64</td>
<td>60</td>
<td>0.83</td>
<td>74</td>
<td>41</td>
<td>0.02</td>
</tr>
<tr>
<td>Positive lymph node status</td>
<td>42</td>
<td>51</td>
<td>0.55</td>
<td>58</td>
<td>36</td>
<td>0.17</td>
</tr>
<tr>
<td>Histologic grade 3</td>
<td>40</td>
<td>77</td>
<td>0.0002</td>
<td>74</td>
<td>82</td>
<td>0.74</td>
</tr>
<tr>
<td>Estrogen receptor negative</td>
<td>11</td>
<td>89</td>
<td>0.0001</td>
<td>84</td>
<td>95</td>
<td>0.38</td>
</tr>
<tr>
<td>Progesterone receptor negative</td>
<td>16</td>
<td>91</td>
<td>0.0001</td>
<td>87</td>
<td>95</td>
<td>0.39</td>
</tr>
<tr>
<td>HER-2 amplification</td>
<td>13</td>
<td>23</td>
<td>0.30</td>
<td>35</td>
<td>5</td>
<td>0.0009</td>
</tr>
<tr>
<td>EGFR overexpression</td>
<td>0</td>
<td>34</td>
<td>0.0001</td>
<td>26</td>
<td>45</td>
<td>0.15</td>
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<tr>
<td>EGFR amplification</td>
<td>0</td>
<td>4</td>
<td>0.50</td>
<td>6</td>
<td>0</td>
<td>0.51</td>
</tr>
<tr>
<td>Vimentin-positive</td>
<td>2</td>
<td>49</td>
<td>0.0001</td>
<td>32</td>
<td>73</td>
<td>0.005</td>
</tr>
<tr>
<td>c-kit-positive</td>
<td>0</td>
<td>36</td>
<td>0.0001</td>
<td>23</td>
<td>55</td>
<td>0.02</td>
</tr>
<tr>
<td>Bcl-2 negative</td>
<td>7</td>
<td>66</td>
<td>0.0001</td>
<td>68</td>
<td>64</td>
<td>0.78</td>
</tr>
<tr>
<td>p53 overexpressed</td>
<td>11</td>
<td>46</td>
<td>0.0002</td>
<td>52</td>
<td>32</td>
<td>0.39</td>
</tr>
<tr>
<td>p63 positive</td>
<td>7</td>
<td>11</td>
<td>0.50</td>
<td>13</td>
<td>9</td>
<td>0.99</td>
</tr>
<tr>
<td>CK8/18 positive (≥5%)</td>
<td>Not determined</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>1.00</td>
</tr>
<tr>
<td>CK17</td>
<td>Not determined</td>
<td>32</td>
<td>—</td>
<td>52</td>
<td>5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Heterogeneous (5-69%)</td>
<td>47</td>
<td>45</td>
<td>—</td>
<td>52</td>
<td>5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Uniform (≥70%)</td>
<td>21</td>
<td>3</td>
<td>—</td>
<td>45</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*5% to 100% of CK5/14-positive carcinoma cells.
†5% to 69% of CK5/14-positive carcinoma cells.
‡70% to 100% of CK5/14-positive carcinoma cells.

Tumors were larger but less frequently vimentin- and c-kit – positive than the basal tumors (P = 0.02, P = 0.005, and P = 0.02, respectively; Table 1). All tumors showed positive staining for CK8/18 (≥5% positive tumor cells) regardless of CK5/14 subtype (Table 1). Additionally, both basal and basoluminal tumors showed uniform staining pattern for CK8/18 (95% and 90%, respectively; P = 0.63). A significant association was observed between the basoluminal tumors and HER-2 oncogene amplification (P = 0.009; Table 1). In fact, the prevalence of HER-2 positivity decreased almost linearly with the increase in the proportion of CK5/14-positive tumor cells (Fig. 2). There was no significant difference in the prevalence of HER-2 amplification between basoluminal tumors and luminal estrogen receptor – negative tumors (35% versus 59%; P = 0.11). In this cohort, there were two tumors with EGFR amplification; both belonged to the basoluminal tumor subtype (Table 1).

The median Ki-67 labeling index was 33% in the basoluminal tumors, in contrast to 58% in the basal tumors (P = 0.0014; Fig. 3A). When grouped together, tumors expressing basal cytokeratins showed a higher cell proliferation activity than the luminal estrogen receptor – negative grade 3 tumors (median 37% versus 22%; P = 0.003; Fig. 3A).

The cell proliferation activity of the CK5/14-positive and CK5/14-negative tumor cell populations in basoluminal tumors was studied with immunohistochemical double staining [CK5/14 using AEC (red) and Ki-67 using 3,3’-diaminobenzidine (brown); Fig. 3B]. The Ki-67 labeling indices in the two cell populations strongly correlated in the individual tumors (r = 0.60, P = 0.0017; Fig. 3C); however, the CK5/14-negative tumor cells had a slightly higher proliferation activity than the CK5/14-positive tumor cells (median 30% versus 19%; P = 0.04).

**Relapse-free survival of the basal and basoluminal tumors.** The relapse-free survival of the CK5/14-positive tumors and their subtypes was studied in a cohort of 382 high-risk breast cancer patients from the Scandinavian Breast Group 9401 study. We found 73 (19%) tumors with at least 5% of the tumor cells showing CK5/14 expression. As shown in Fig. 4A, more recurrences were observed in the CK5/14-positive tumor group during the initial years of follow-up; however, by the end of the follow-up, these differences were not observed (P = 0.52). The efficacy of the chemotherapy regimes (tailored and dose-escalated versus standard 5-fluorouracil, epirubicin, and cyclophosphamide followed by cyclophosphamide, thiotepa, and carboplatin along with bone marrow support) was not associated with basal cytokeratin expression (data not shown).

The relapse-free survival of CK5/14-positive tumor subtypes, i.e., basal and basoluminal, substratified by HER-2 status is shown in Fig. 4B. The basoluminal tumors showed shorter relapse-free survival than basal tumors (P = 0.01). The substratification of the basoluminal group by HER-2 status (P > 0.05) shows that the survival difference between basal and basoluminal tumors is not due to HER-2. The subgroup characterized by uniform basal cytokeratin expression (basal) and HER-2 amplification was too small (n = 2) to be included in the survival analysis. For one basal tumor, HER-2 status was not known.
Thus far, basal cytokeratin expression has been considered to characterize mainly a single entity of breast tumors (1, 3, 5, 8, 12–14, 27–30). We divided basal cytokeratin–positive breast carcinomas into two subtypes based on the intratumoral heterogeneity, i.e., into tumors with 5% to 69% or \( \geq 70\% \) CK5/14-positive tumor cells. In tumors with a low proportion of CK5/14-positive tumor cells, the staining often showed a checkerboard pattern with strongly CK5/14-positive and totally negative cells located next to each other. All tumors in both subtypes showed luminal CK8/18 positivity. Thus, in heterogeneously CK5/14-positive tumors, a large number of tumor cells express luminal cytokeratins only. Based on this observation, these tumors were called basoluminal tumors, in contrast to the uniformly CK5/14-positive basal tumors. The classification based on CK5/14 was confirmed by the immunohistochemical analysis of CK17, which is the third major basal epithelium cytokeratin expressed in breast tumors (21). The basal cytokeratin staining types (basoluminal and basal) also seem to persist during the metastatic progression, implying that this phenotype might be determined during the early phase of tumor development.

The biological correlates of the basoluminal and basal tumor subtypes were tested by using clinical and biological markers that have been associated with basal cytokeratin expression. Although both subtypes clearly shared the major features of the basal cytokeratin–positive tumors (hormone receptor negativity and high tumor grade), the basal tumors were more often positive for vimentin and c-kit, which suggests that the uniformly CK5/14-positive basal tumors represent an entity that more closely resembles the primitive breast epithelium progenitor cell (14, 16, 19, 31, 32). The basoluminal tumors may represent an entity that is intermediate between uniformly CK5/14-positive basal tumors and totally CK5/14-negative luminal tumors. In line with the phenotypic tumor markers, the basal cytokeratin–positive tumor subgroups showed differences in cell proliferation activity (Ki-67 labeling index). On average, Ki-67 indices were highest in basal tumors, followed by basoluminal tumors and grade 3 breast cancers showing CK5/14-negative and estrogen receptor–negative phenotype. This also suggests a differential biology for the basal and basoluminal tumors. Interestingly, in double immunostaining of CK5/14 and Ki-67, the Ki-67 labeling indices of CK5/14-positive and CK5/14-negative cells correlated closely in the basoluminal tumors. Thus, cell proliferation activity is an inherent feature of the tumor, and does not seem to be directly dependent on whether the cancer cell is CK5/14-positive or not in the basoluminal tumor.

Gene expression microarrays have almost exclusively been able to discriminate basal-like tumors and HER-2 oncogene amplified tumors as separate clusters (1, 10–12). The inverse association is not complete because basal cytokeratin expression and HER-2 oncogene amplification have been found concomitantly in tumors (2, 8) and in one breast cancer cell line (33). HER-2 oncogene amplification was found almost exclusively in the basoluminal tumors and not in basal tumors. In line with HER-2, EGFR gene amplification was observed only
in basoluminal tumors. This indicates different oncogenic activation pathways for basoluminal and basal tumors. A possible explanation for the distinct classification of HER-2 amplified tumors and basal-like tumors in the microarray studies (1, 10–12) is that the microarrays may actually identify only those tumors with uniform basal cytokeratin expression as a separate basal-like tumor cluster. Our finding of basal cytokeratin expression in a minority of cancer cells may also provide an explanation for tumors identified as CK5 and CK17 mRNA-negative in the second basal-like cluster in the gene expression microarray study by Perou et al. (1). In these tumors, basal cytokeratin mRNAs might be too low to be detected as overexpressed in tumor tissue homogenates. Thus, gene expression profiling and microarray-based classification of the basal and basoluminal subtypes, as defined immunohistochemically in our study, remains to be studied.

In previous immunohistochemical studies, no consensus of an optimal cutoff for classifying a tumor as basal cytokeratin-positive had been reached. Cutoffs ranging from any basal cytokeratin immunoreactivity (>0%) to 5%, or up to 20% positive cells have been used (2, 3, 5, 7, 12, 13). As shown by our results, the selected cutoff is likely to affect the clinicopathologic and survival correlations. When the cutoff was set at 5% of positive tumor cells, the identified tumors still show the typical characteristics of the basal phenotype tumors, such as estrogen receptor negativity, high tumor grade, and high proliferative activity (2–9), but also features that are not typical to basal-like tumor cluster according to gene expression microarray studies (HER-2 amplification; refs. 1, 10–12). When the cutoff is set at 70% of positive tumor cells, a more homogeneous group of tumors is identified; this group is also strongly associated with vimentin, c-kit, and no HER-2 amplification. If the latter classification is used, the basal phenotype tumor subgroup would comprise only 4% to 5% of all invasive breast cancers (22 of 506 in our study). A schematic presentation of the basal, basoluminal, and luminal subtypes and their clinicopathologic characteristics is represented in Fig. 5.

Previous studies using immunohistochemistry have shown that basal cytokeratin immunoreactivity in sporadic breast tumors is associated with poor prognosis (7, 8, 12, 27). We studied a patient population of high-risk breast cancers for relapse-free survival after adjuvant chemotherapy. In this cohort, we could not see a significant difference in relapse-free survival between CK5/14-negative and CK5/14-positive tumors. This might be due to the selection of patients with high-risk breast cancer (having at least five positive lymph nodes) to the study. Furthermore, it is possible that the intensive chemotherapies given to all the patients may have obscured survival differences found in conventionally treated patients (8, 27).

Supporting our classification of basal cytokeratin-positive tumors into basal and basoluminal subtypes, we observed that basoluminal tumors showed shorter relapse-free survival than the basal tumors. The basoluminal tumor group was stratified by HER-2 status to show that the difference between basal and basoluminal tumors was not due to more frequent amplification of HER-2 in the basoluminal group. Thus, it is likely that basal and basoluminal tumors differ in their natural biological aggressiveness. Alternatively, it is possible that these subgroups differ with regard to responsiveness to...
chemistry. We consider this less likely because in this material, CK5/14 was not associated with relapse-free survival in patient groups randomized to receive cyclophosphamide, thiotepa, and carboplatin or dose-escalated 5-fluorouracil, epirubicin, and cyclophosphamide treatments.

In this study, we have shown that half of the basal cytokeratin–positive tumors comprised a mixture of CK5/14-negative and CK5/14-positive tumor cells. This supports their origin from premature progenitor cell, which is capable of differentiating into both luminal and myoepithelial lineages in a normal breast (14, 19, 20). As the basoluminal tumors show both mature cells (CK5/14– CK8/18+) and more immature cells (CK5/14+ CK8/18+), it is possible that differentiation into the luminal phenotype can also occur after malignant transformation. Whether this type of differentiation is an inherent feature of the parental cell or an effect of genetic instability, remains to be elucidated. Our observation that heterogeneous CK5/14 expression (basoluminal type) was also found in metastases suggests that genesis of CK5/14-positive and CK5/14-negative tumor cells might occur constantly throughout every cell generation. The persistence of both CK5/14-negative and CK5/14-positive tumor cells in the metastases also suggests that CK5/14 expression in basoluminal tumors probably does not provide significant advantage in metastatic dissemination and growth.

In conclusion, we propose a new tumor classification based on the intratumoral heterogeneity of basal cytokeratin expression. The basoluminal and basal tumor subtypes show significant differences in cell proliferation activity, biomarker and oncogene profile, and patient survival.

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Clinical Cancer Research

Basoluminal Carcinoma: A New Biologically and Prognostically Distinct Entity Between Basal and Luminal Breast Cancer

Mervi Laakso, Minna Tanner, Jonas Nilsson, et al.


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