‘Accidental’ anti-angiogenic drugs: anti-oncogene directed signal transduction inhibitors and conventional chemotherapeutic agents as examples

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Abstract

A number of drugs currently being tested in clinical trials as possible angiogenesis inhibitors were not originally developed with the intention of suppressing tumour angiogenesis. Thalidomide and interferon alpha are obvious examples of such drugs. This list of ‘accidental’ angiogenesis inhibitors may include established agents such as conventional cytotoxic chemotherapeutic drugs as well as the new generation of anticancer drugs known as anti-oncoprotein signal transduction inhibitors. With respect to the former, the potential of such drugs to inhibit angiogenesis could be the result of their ability to cause collateral damaging effects on cycling endothelial cells found in newly formed blood vessels, or inhibiting other vital endothelial cell functions necessary for angiogenesis. The antitumour vascular side-effects of chemotherapy may be optimised by administering such drugs continuously on a more frequent (e.g. weekly or even daily) basis at levels well below the maximum tolerated dose (MTD), especially when this is done in combination with newly developed anti-angiogenic drugs such as vascular endothelial cell growth factor (VEGF) receptor blocking antibodies. This strategy may minimise or delay the problems of host toxicity and acquired drug resistance. The possibility of anti-angiogenic effects mediated by signal transduction inhibitors such as ras farnesyltransferase inhibitors (ras FTI’s), or drugs which block receptor tyrosine kinases (e.g. ErbB2/neu) such as Herceptin, may be the consequence of such oncogenes inducing or upregulating various pro-angiogenic molecules such as VEGF (vascular endothelial cell growth factor) in tumour cells. Hence, treatment of tumour cells with such drugs can lead to downregulation of tumour cell-associated VEGF expression and this can contribute to an anti-angiogenic effect of the drug in vivo. In addition, some of these drugs may also affect certain ‘activated’ endothelial cell functions directly so as to block angiogenesis. An awareness of the potential of such conventional or experimental anticancer drugs to affect tumour growth through blockade or suppression of angiogenesis has implications for how anticancer drugs may be used clinically, either alone, or in combination with other drugs to optimally treat cancer. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

There are a number of reasons for the current preclinical and clinical interest in developing anti-angiogenic drugs to treat cancer [1] — a concept first proposed by Folkman [2]. One is the molecular elucidation of a number of pro-angiogenic growth factors, such as vascular endothelial cell growth factor (VEGF), the angiopoietins, and the receptor tyrosine kinases expressed by activated endothelial cells of newly formed vessels for such growth factors [3]. These and other discoveries have provided targets for the rational development of a number of anti-angiogenic drugs such as anti-VEGF neutralising antibodies [4] and agents which block receptors for VEGF or the angiopoietin [1,5–8]. A second factor for the current popularity of tumour angiogenesis research is the realisation that some anti-angiogenic drugs may delay or even circumvent the problem of acquired drug resistance [9,10] based on the fact that they target the genetically stable endothelial...
cells of newly formed tumour blood vessels, rather than genetically unstable tumour cells per se which are much more prone to mutate and develop resistance because of such genetic instabilities [11,12].

In addition to rationally designed anti-angiogenic drugs, there is a remarkably diverse group of angiogenesis inhibitors being tested that were not originally designed to function as such, e.g. thalidomide, interferon-alpha and interleukin-12 [1,13]. Perhaps of even greater interest is that this list may include many, if not all, conventional cytotoxic chemotherapeutic drugs [1], radiation [14] and hormonal ablation therapies [13,15]. It may also include the new generation cytostatic anticancer drugs such as signal transduction inhibitors designed to block the function of various oncoproteins [16] such as Herceptin and ras farnesyltransferase inhibitors [16]. With respect to chemotherapy, the presence of dividing endothelial cells in newly forming tumour blood vessels [17–19] may render such vessels — in contrast to their mature, quiescent counterparts found in normal adult tissues — relatively sensitive to the cytotoxic effects of such drugs in a manner similar to dividing bone marrow, hair follicle or gut mucosal cells [11]. This hypothesised ‘collateral damage’ to the tumour vasculature could conceivably contribute to the antitumour efficacy of chemotherapy in vivo or other agents as first proposed by Denekamp in 1982 [19,20]. If true, it should follow, as we first proposed in 1991 [11] that even tumours consisting of tumour cells resistant to a particular drug might still respond to that drug through such anti-vasculature ‘side-effects’. However, this presumably occurs infrequently, since most human cancers are intrinsically resistant to chemotherapy, or initially respond, only to recur as a result of the overgrowth of drug resistant subpopulations. This is puzzling, especially given recent reports from several groups showing that a variety of conventional chemotherapeutic drugs can bring about significant anti-angiogenic or anti-vascular effects in vivo in a number of assays of angiogenesis [1,21]. These drugs include paclitaxel, camptothecin analogues and vinca alkaloids, among others [22–24], as summarised in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Reports of anti-angiogenic effects mediated by conventional chemotherapeutic drugs</th>
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<tbody>
<tr>
<td><strong>Anti-metabolites</strong></td>
</tr>
<tr>
<td>1. Inhibition of in vitro vascular endothelial cell proliferation and in vivo neovascularisation by low-dose methotrexate [26].</td>
</tr>
<tr>
<td>2. Purine analogue 6-methylmercaptopurine riboside inhibits early and late phases of the angiogenic process [97].</td>
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<tr>
<td><strong>Taxanes</strong></td>
</tr>
<tr>
<td>1. The microtubule-affecting drug paclitaxel has antiangiogenic activity [22].</td>
</tr>
<tr>
<td>2. Inhibition of angiogenesis and breast cancer in mice by the microtubule inhibitors 2-methoxyoestradiol and paclitaxel [98].</td>
</tr>
<tr>
<td>3. Inhibition of tumour angiogenesis and induction of apoptosis as properties of docetaxel [99].</td>
</tr>
<tr>
<td><strong>Camptothecin and analogues</strong></td>
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<tr>
<td>2. Anti-angiogenic potential of camptothecin and topotecan [36].</td>
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<tr>
<td><strong>Anthracyclines</strong></td>
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<tr>
<td>1. Inhibition of angiogenesis by anthracyclines and titanocene dichloride [100].</td>
</tr>
<tr>
<td>2. Antiangiogenic chemotherapeutic agents: characterisation in comparison to their tumour growth inhibition in human renal cell carcinoma models [101].</td>
</tr>
<tr>
<td><strong>Alkylating agents</strong></td>
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<td>Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug resistant cancer [25].</td>
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2. **Low-dose continuous chemotherapy as a feasible anti-angiogenic strategy**

Recent results from Folkman’s laboratory have helped shed some light on the above paradox [25]. Chemotherapy is normally given acutely, usually in the form of bolus infusions at maximum tolerated doses (MTD) with long rest periods (e.g. 3 weeks) between successive drug exposures. It has been suggested that these rest periods provide the endothelial cell compartment of a tumour an opportunity to repair some of the damage inflicted by the chemotherapy [25]. Browder and colleagues have recently proposed that this repair process could be partially compromised by administering lower doses of a chemotherapeutic drug, such as cyclophosphamide, more frequently, e.g. once a week. Indeed, there is some limited evidence that low-dose continuous methotrexate chemotherapy can cause significant anti-angiogenic effects [26]. This ‘anti-angiogenic scheduling’ of chemotherapy [25] optimises anti-angiogenic ‘side-effects’ of chemotherapy such that even a sub-line of the Lewis Lung Carcinoma previously selected in vivo for acquired resistance to the MTD of cyclophosphamide can be rendered sensitive again to the drug in vivo by changing to a continuous low-dose therapy protocol of the same drug [25].

We decided to test the effects of low-dose continuous chemotherapy as a possible anti-angiogenic strategy, but with one important modification, namely, using it in combination with an agent which blocks the function of...
VEGF receptor-2 (flk-1/KDR), and hence VEGF itself. The rationale for testing this particular combination treatment strategy is based on the finding that a major function of VEGF is now recognised to be promotion of survival of endothelial cells comprising newly formed vessels [15,27,28]. Hence the ability of such cells to cope with the damage inflicted by continuous low-dose exposures to a chemotherapeutic drug could be selectively and significantly impaired, given the highly restricted pattern of expression of VEGF receptors to activated endothelial cells [3,6,7]. This could reduce host toxicity and thereby allow for longer-term administration of the chemotherapeutic agent without necessarily sacrificing and perhaps even improving, antitumour efficacy.

For our initial experiments, we used vinblastine, a monoclonal anti-flk-1 neutralising antibody called DC101 [5,6], to treat poor prognosis related human neuroblastoma cell lines grown as xenografts in SCID mice, to test the effectiveness of this combination treatment strategy [29]. Poor prognosis neuroblastoma in children is usually treated with aggressive combination chemotherapy at MTDs, with or without a bone marrow transplant [30]. However, such therapies are associated with severe side-effects and are seldom effective. Therefore, we designed experiments to determine if it was possible to develop a more acceptable treatment alternative, and one that could also be applied to other types of cancer as well.

Xenografts of two independent neuroblastoma cell lines (SK-N-MC and SK-N-AS) were subjected to either continuous treatment with low-doses of vinblastine, the monoclonal DC101 neutralising antibody, or both agents together. Details are described in Fig. 1, and elsewhere [29]. Similar to DC101, we found that low-dose vinblastine treatment resulted in significant but ultimately temporary xenograft regressions (see Fig. 1); we also found evidence for a decrease in tumour vascu-

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**Fig. 1.** Induction of solid tumour regression by non-toxic, anti-angiogenic combination therapy with low-dose vinblastine (Vb1), and anti-flk-1/VEGFR2 antibody (DC101). (a) Established xenografts of human neuroblastoma (SK-N-MC) were treated by antivascular regimen of low-dose vinblastine (induction: 0.75 mg/m² bolus intraperitoneally (i.p.), 1 mg/m²/day continuous subcutaneous (s.c.) infusion for 3 weeks, maintenance: 1.5 mg/m² every 3 days) alone or in combination with an anti-VEGFR2 antibody (DC101; 800 μg every 3 days) or DC101 alone was used. There is an appreciable tumour growth inhibition by each of the single agents, which is comparable, at least initially, with that of the combination treatment group. The benefit of the combination treatment is most evident after prolonged treatment, when lasting and complete tumour regression is observed. The data is a compilation of two independent experiments, initial experiment lasting 34 days and the follow-up which is still ongoing (>210 days). In both sets, 20 mice were randomised into four groups. (b) Lack of toxicity-dependent weight loss in mice bearing SK-N-MC tumour xenografts treated with ‘antivascular’ vinblastine regimen alone or in combination with and anti-VEGFR2 (DC101) antibody or DC101 antibody used alone. There are no significant differences in weight between the groups, except for a transient (14–18 days) episode of weight loss associated with diarrhoea in the combination treatment group. The episode resolved without interruption of therapy. Average body weights (g) +/- S.D. are plotted (n=3–10 mice). Taken from Klement and colleagues [29].
larity, and direct inhibition of angiogenesis as assessed by the Matrigel plug assay [29]. In marked contrast, the combination therapy resulted in full and sustained regressions of large (0.75 mm³) established tumours, without an ensuing increase in host toxicity or any signs of acquired drug resistance during the course of continuous treatment, which lasted for over 6 months (Fig. 1; [29]). These results, along with those of Browder and colleagues [25], implicate the potential benefits of using such a chronic chemotherapy-based strategy as a possible clinical treatment to target the endothelial cell compartment of tumours with high efficacy and low host toxicity.

It should be noted that interest in the therapeutic effects of combining anti-angiogenic drugs with conventional chemotherapy was initially stimulated by reports from Teicher’s laboratory [31] showing that the antitumour effects of MTDs of various chemotherapeutic cytotoxic drugs can be augmented, at least in preclinical experiments, when they are combined with angiogenesis inhibitors [32]. Counterintuitively, such effects have been attributed to greater delivery of the cytotoxic drugs into tumour masses [32]. Increasing the efficacy of cyclic high-dose chemotherapy in such a manner, however, would unlikely alter two of the major problems associated with the use of chemotherapy given in this way: induction of moderate to severe side-effects, (e.g. myelosuppression, nausea, vomiting and hair loss), and the eventual development of acquired resistance to the cytotoxic drugs. In contrast, our results suggest that these problems can be significantly attenuated, and perhaps even avoided, by using much more frequent administration of significantly lower doses of a chemotherapeutic drug, e.g. vinblastine, when given in combination with anti-VEGF receptor-2 neutralising antibodies, without sacrificing antitumour efficacy. The dose of vinblastine used in our experiments was in the range of 1.5 mg/m², every 3 days, which is approximately 1/4 of the MTD of this drug in humans, and 1/16–1/20 of the MTD in mice, given the fact that the MTD of vinblastine in mice is four to five times higher than in humans [33,34]. Similar to Browder and colleagues who used low-dose cyclophosphamide therapy [25], we found evidence, using the Matrigel plug assay, that continuous low-dose vinblastine administration can cause a direct anti-angiogenic effect in vivo.

While our results are consistent with the hypothesis that the anti-vascular effects of vinblastine are significantly enhanced by combination with anti-VEGF R2 (flk-1) antibodies, other, or even different, mechanisms may be involved. In this regard, it is important to note that extremely low (e.g. picomolar) doses of vinblastine that are devoid of endothelial cytotoxicity can still block aspects of angiogenesis in vitro or in vivo, using the CAM (chorioallantoic membrane) assay [35]. Similarly, low (nanomolar) and non-cytotoxic concentrations of camptothecin and topotecan can block endothelial cell functions in vitro that are relevant to angiogenesis [36]. The same appears true for paclitaxel [22]. Thus, other functions such as endothelial cell motility, invasion, protease production and vessel remodelling may be altered by low-dose chemotherapy [35], the extent of which could be significantly enhanced by simultaneous exposure to anti-VEGF receptor-2 antibodies.

The results of Vacca and coworkers and Browder and colleagues [25,35] as well as ours, also raise the important question of what the optimum low-dose of a given chemotherapeutic drug is with respect to inducing anti-angiogenic effects. An analogous situation has been described recently by Fidler and colleagues with respect to interferon alpha as an anti-angiogenic agent to treat experimental tumours [37]. Thus, whereas very high-doses of interferon alpha (e.g. 70 000 units) given on weekly basis had little effect, much lower-doses (e.g. 10 000 units) given on a daily basis strongly inhibited tumour growth by an anti-angiogenic mechanism [37]. Such results highlight what could be the difficulty in selecting the optimal therapeutic dose of certain new anti-angiogenic drugs, for both preclinical studies and clinical trials.

We would also note that there is increasing use of clinical chemotherapy protocols employing lower-doses of drug (e.g. docetaxel) given frequently, e.g. weekly, especially as a means of minimising toxic side-effects, such as myelosuppression [38], as well as increasing use of oral chemotherapy [39]. Such developments clearly make it timely to test clinically low-dose regimens of conventional chemotherapeutic drugs alone or in combination with certain newly developed angiogenesis inhibitors, as described here, that can be given continuously, perhaps even on a daily basis [40], without significant toxic side-effects, and which are not rapidly rendered ineffective by the development of acquired drug resistance. This prospect also clearly increases the need to begin evaluating in-depth optimal ‘anti-vascular’ dosing and scheduling characteristics of different chemotherapeutic drugs, and to test the effects of such therapeutic approaches in different preclinical tumour models as well as in appropriately designed clinical trials.

3. Oncogenes and tumour angiogenesis: signal transduction inhibitors as anti-angiogenic drugs

There are two major types of genetic alteration which, together, can result in the sequential changes associated with the long-term transition of normal cells and tissues to malignant cancers. These are the activation of dominantly-acting oncogenes, such as ras, and inactivation or loss of expression of recessive tumour suppressor genes, such as TP53 [41]. Most of these genetic
changes endow the cells which harbour them with a selective growth advantage. The way in which they do so has been generally considered to be through direct aberrations involving the molecular machinery controlling cell proliferation [41]. However, over the past decade there has been a shift in thinking which has resulted in an increased emphasis on the contribution that such genetic changes make to the enhanced survival of tumour cells, by virtue of their ability to cause suppression of programmed (apoptotic) cell death. In particular, the discovery of the anti-apoptotic effects of the bcl-2 or bcl-X<sub>1</sub> proto-oncogenes [42–45] and mutant TP53 [46] were among the key discoveries which have helped fuel interest in the role of apoptosis, or more specifically its suppression, in cancer development and growth [47–49].

There is, however, a potentially powerful but indirect way in which oncogenes and (inactivated) tumour suppressor genes may contribute to the proliferative and survival advantage of cancer cells, and that is through their effects on stimulating tumour angiogenesis.

The idea that oncogenes and tumour suppressor genes contribute to tumour angiogenesis was first hypothesised by N. Bouck [50], and was based on initial findings that non-tumorigenic somatic cell hybrids created between tumorigenic cells and normal cells secreted a factor — later identified to be thrombospondin-1 [51,52] — that inhibited angiogenesis [51,52]. It is known that such non-tumorigenic somatic cell hybrids eventually acquire tumorigenic competence as a result of the loss of a particular chromosome from the normal cell partner that carries a putative wild-type tumour suppressor gene. Thus, Bouck’s results implicated the possibility that wild-type tumour suppressor genes may, at least in part, inhibit tumour growth indirectly, as a result of suppression of angiogenesis [51]. Subsequently wild-type TP53 was shown to be a transcriptional activator of thrombospondin-1; thus, loss of TP53 expression could result in its suppressed production [52]. This in turn would facilitate the pro-angiogenic potential of various peptide growth factors such as VEGF. Indeed, Bouck’s results were instrumental in the formulation of the idea that tumour angiogenesis is switched on as a result of a change in the net balance of angiogenesis stimulators to angiogenesis inhibitors, in favour of the former [51,53].

Historically stimulation of the production of mitogenic peptide growth factors in cancer cells has been considered to be one of the main ways in which oncogenes contribute to the development of cancer. Most of these growth factors, such as the transforming growth factor-alpha (TGF-α), are thought to act in an autocrine fashion to directly stimulate tumour cell proliferation, by virtue of expression, by the same tumour cells, of receptors for these oncogene-encoded growth factors. However, some of these autocrine growth factors, such as basic fibroblast growth factor (bFGF), and TGF-α, can also act in a paracrine fashion to stimulate endothelial cell growth, migration, and survival, i.e. angiogenesis [51,54]. Moreover, there are certain growth factors, such as VEGF, that have no known autocrine mitogenic effects, but which can, in a paracrine manner, stimulate tumour growth in a powerful but indirect manner, by promoting the growth of adjacent new blood vessel capillaries [55,56].

The expression of VEGF is often upregulated in tumour cells, and this may be due, at least in part, to hypoxia which is a common feature of solid tumours [55,57,58]. Hypoxia can stimulate VEGF expression transcriptionally and by increasing the stabilisation of VEGF mRNA [59,60]. But is it not possible that some of the genetic changes associated with the development of cancer, e.g. oncogenic ras mutations, also serve to upregulate VEGF expression? Our interest in examining this question stemmed in part from an experimental paradox we had noticed with respect to the mode of action of small molecule drugs, i.e. ras farnesyltransferase inhibitors (ras FTIs), which have been used to block the growth of ras oncogene expressing tumours [61]. These drugs were at first thought incapable of killing tumour cells in cell culture, instead they block cell proliferation [62,63]. But in certain tumour models, e.g. breast or salivary gland tumours arising in H-ras transgenic ‘oncomice’, treatment with such drugs was found to cause acute regressions of large established tumours which was not consistent with the drug having only cytostatic properties in vivo [61]. We reasoned the lack of a cytotoxic effect in cell culture could be due to the use of inappropriate assay conditions, i.e. use of monolayer cell cultures, and predicted that under three-dimensional, anchorage-independent conditions ras oncogenes may actually have a survival function. This is because normal epithelial cells normally undergo a form of apoptosis called ‘anoikis’ when forced to grow anchorage-independently, a phenomenon which can be prevented by oncogenes or mutant/inactivated suppressor genes such as ras and TP53, respectively, such a pro-survival function would, therefore, be compromised by exposure to a ras FTI [64]. This was subsequently found to be the case, both in vitro [65] and in vivo [66]. Thus, blocking ras protein function may cause direct apoptosis of tumour cells, at least in some circumstances.

We also reasoned that blocking the function of ras oncogenes might also cause some tumour cell death through an indirect mechanism, namely by inhibition of angiogenesis. In theory, this could occur if ras mutations result in induction or upregulation of pro-angiogenic growth factors such as VEGF. In fact this was first shown in 1995 by our laboratory [67] and Marme’s group [68]. If this hypothesis is correct, then blocking ras oncogene encoded (or related) proteins with a ras FTI, or similar-acting drug, could result in suppressed
VEGF expression, and hence, possible inhibition of tumour angiogenesis. However, this would not lead to any antitumour therapeutic effect in vitro — where angiogenesis is not required. In contrast, it could do so in vivo. Moreover, this could even result in some degree of vessel regression since VEGF can act as a survival factor for the endothelial cells of newly formed immature blood vessel capillaries [27] and hence secondary death of tumour cells immediately adjacent to such regressing vessels [69].

Subsequent studies by us showed that ras mutations were associated with strong VEGF induction, both in rodent and human intestinal epithelial cells [67]. Moreover, treatment of H-ras transformed rat intestinal epithelial cells in vitro with a ras FTI caused a significant reduction in VEGF expression [67]. A large number of studies have now shown that ras mutations can lead to induction of VEGF expression in a diverse and broad array of experimental systems as summarised in Table 2, in both animal and human cell lines [68,70–78], and that ras FTIs can block VEGF production in treated tumour cells in vitro as well as angiogenesis [67]. Indeed, Gu and coworkers have also found that ras FTIs can block angiogenesis directly, by inhibiting certain endothelial cell functions critical to angiogenesis [79].

The stimulatory effect of ras oncogenes on VEGF expression in cell culture is not restricted to this category of oncogenes. For example, overexpression and/or activation of the EGF, erbB2/Her-2/neu or IGF-1 receptor tyrosine kinases is also associated with VEGF induction or upregulation [80–82]. Moreover, similar to the results we obtained with a ras FTI, treatment with monoclonal neutralising antibodies directed to the human EGF or erbB2/Her-2/neu receptor tyrosine kinases can also result in suppression of VEGF mRNA and protein expression in appropriate target tumour cells in vitro [80]. Of considerable interest, this effect could be duplicated in vivo on a human tumour xenograft (A431 squamous carcinoma cells) in which the tumour-bearing mice were treated with the chimeric anti-human EGF receptor (‘C225’) monoclonal neutralising antibody [80]. Whether the suppression of VEGF expression observed after such treatment contributes to the antibody’s antitumour effects in vivo, and to what extent, remains to be established. Other types of oncogenes which can stimulate VEGF production include non-receptor tyrosine kinases such as v-src [83], transcription factors such as c-jun and c-fos [84,85] and human papilloma virus (HPV)-16 encoded oncoproteins such as E6 [86].

It is also noteworthy that hypoxia and oncogene expression may cooperate in a synergistic manner to maximally stimulate VEGF expression [71,80]. For example, immortalised NIH-3T3 fibroblasts or IEC-18 rat intestinal epithelial cells do not synthesise significant amounts of VEGF in cell culture, even when exposed to a hypoxia mimetic drug, cobalt chloride [80]. But if oncogene transfectants of such cells are exposed to this drug, the amount of VEGF made — which is already significantly elevated underoxic conditions — can be enhanced even more [80].

In summary, the results suggest that a major and hitherto unappreciated function of a variety of oncogenes is to induce or upregulate the expression of a potent pro-angiogenic growth factor such as VEGF. Other growth factors such as bFGF, TGFz and IL-8 which possess both mitogenic autocrine and paracrine/pro-angiogenic growth promoting effects could be stimulated by dominantly-acting oncogenic changes in a similar way [87]. Furthermore, it is also possible that oncogenes could contribute to tumour angiogenesis in another way, namely, by downregulating the expression of endogenous angiogenesis inhibitors. Indeed, there is evidence that TSP-1 expression is strongly suppressed in ras transformed cells [88,89]. Perhaps other such endogenous inhibitors, e.g. interferon alpha [90–92], might be affected in a similar manner. In other words, just as mutation/inactivation of tumour-suppressor genes can

### Table 2

Reports showing ras oncogene induction or upregulation of VEGF

<table>
<thead>
<tr>
<th>Cells/system</th>
<th>Author [Ref.]</th>
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<tbody>
<tr>
<td>H-ras-transformed intestinal epithelial cells.</td>
<td>Rak and colleagues, 1995 [67]</td>
</tr>
<tr>
<td>v-ras-Transformed NIH-3T3 cells.</td>
<td>Grugel and colleagues, 1995 [68]</td>
</tr>
<tr>
<td>H-ras in mouse squamous cell carcinomas.</td>
<td>Larcher and colleagues, 1996 [72]</td>
</tr>
<tr>
<td>H-ras-transformed NIH-3T3 cells.</td>
<td>Mazure and colleagues, 1996 [71]</td>
</tr>
<tr>
<td>H-ras-transformed endothelial cells.</td>
<td>Arbisier and colleagues, 1997 [102]</td>
</tr>
<tr>
<td>H-ras in hamster buccal pouch keratinocytes.</td>
<td>Lingen and colleagues, 1997 [75]</td>
</tr>
<tr>
<td>H-ras in Li Fraumeni p53-human fibroblasts.</td>
<td>Volpert and colleagues, 1997 [73]</td>
</tr>
<tr>
<td>v-H-ras in human IMR-90 fibroblasts.</td>
<td>Enholm and colleagues, 1997 [77]</td>
</tr>
<tr>
<td>v-H-ras in NIH-3T3 cells.</td>
<td>White and colleagues, 1997 [78]</td>
</tr>
<tr>
<td>v-H-ras transgene in Tg.AC mice.</td>
<td>Tober and colleagues, 1998 [104]</td>
</tr>
<tr>
<td>v-H-ras in NIH 3T3 cells.</td>
<td>Feleszko and colleagues, 1999 [105]</td>
</tr>
<tr>
<td>v126 mutant of c-H-ras in HaCaT human keratinocytes.</td>
<td>Charvat and colleagues, 1999 [106]</td>
</tr>
<tr>
<td>V12G mutant of c-H-ras INK4a−/− mouse melanocytes.</td>
<td>Chin and colleagues, 1999 [107]</td>
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3.12.4.5.1
lead to loss of angiogenesis inhibitors, e.g. suppression of TSP-1 expression in p53 deficient cells [52] or upregulation of pro-angiogenic growth factors, such as VEGF in von Hippel Lindau (VHL)-deficient renal cell carcinoma cells [93,94], similar dual but antithetical events may occur after oncogene activation.

4. Conclusions

The results summarised here are consistent with angiogenesis being a ‘unifying concept of cancer’ [95]. Thus, oncogenes and tumour suppressor genes may contribute to cancer development, at least in part, by inducing angiogenesis and, therefore, drugs designed to exploit such genetic changes may well function, at least inducing angiogenesis and, therefore, drugs designed to contribute to cancer development, at least in part, by angiogenesis being a ‘unifying concept of cancer’ [95].

4. Conclusions

The results summarised here are consistent with angiogenesis being a ‘unifying concept of cancer’ [95]. Thus, oncogenes and tumour suppressor genes may contribute to cancer development, at least in part, by inducing angiogenesis and, therefore, drugs designed to exploit such genetic changes may well function, at least in part, as de facto angiogenesis inhibitors. This notion of accidental angiogenesis inhibitors may even extend to conventional cytotoxic chemotherapeutic drugs and radiation therapy, agents which have been used to treat cancer for over 50 years. Indeed, it may also apply to hormonal ablation therapies and chemoprevention drugs [15,28]. Greater awareness of the collateral damaging effects of many, if not most, anticancer therapeutics should make it possible to optimise this beneficial therapeutic side-effect and exploit its potential advantages to the fullest extent — such as circumventing or delaying the problem of acquired drug resistance and minimising host toxicity. Administration of conventional cytotoxic drugs in a continuous low-dose manner, in combination with certain angiogenesis inhibitors may be an exciting example of this possibility [25,29]. Similarly, the effects of ionising radiation may also be significantly enhanced by cotreatment with antiangiogenic agents such as VEGF neutralising antibodies [96].

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