



Curcumin and its derivatives in breast cancer: Current developments and potential for the treatment of drug-resistant cancers

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Abstract

Curcumin, a spice found in curry powder, is receiving considerable attention as a possible chemopreventative and chemotherapeutic. This review considers the evidence for the use of curcumin as a treatment for breast cancer, particularly triple negative breast cancers (lacking ER, PR and HER2) which are resistant to many current treatments. Evidence suggests that curcumin suppresses the growth of breast cancers both *in vitro* and *in vivo*. In ER-cell lines curcumin causes apoptosis via a range of mechanisms at concentrations ranging between 1 μM and 7.6 μM depending on the cell line and system. In xenograft models, curcumin has been shown to be effective but is limited by its bioavailability. This can be improved by nanotechnologies such as micelles. Studies with micelles have shown that these systems increase the uptake and cytotoxicity of curcumin *in vitro*. In xenotransplantation models micelles improved bioavailability and increased the half-life of curcumin while showing increases in apoptosis in implanted tumours. The last area discussed is the development of curcumin derivatives. Many of these show increased cytotoxicity (less than 1 μM) and improved pharmacokinetic profiles *in vivo*. The most potent of these compounds developed to date are RL66 and RL71 with IC_{50} values less than 1 μM across a range of breast cancer cell lines, decreased metastasis in a xenograft model, decreased angiogenesis markers and improved tumour growth inhibition as compared to curcumin. Overall, this review concludes that curcumin and its derivatives show future potential as a powerful broad-spectrum treatment for breast cancer.

Keywords: Curcumin, breast cancer, nanotechnology, micelle, derivatives, chemotherapeutic, triple negative breast cancer

Introduction

Curcumin is derived from the spice turmeric (or curry powder) and has long been used to enhance the flavour of food. It has been demonstrated to have a wide range of cellular effects including chemopreventative and chemotherapeutic properties. Over the past five years this has led to a deluge of information on the mechanism by which curcumin acts. However, curcumin is poorly absorbed through the gastrointestinal tract. Therefore, a number of laboratories around the world are looking to increase the clinical efficacy of curcumin through chemical modification or through nanotechnology delivery systems. This review focusses specifically on the potential of curcumin and its derivatives as chemotherapeutics for breast cancer. The triple negative subset of breast cancers that do not respond well to current treatments will be afforded particular focus.

Breast cancer

Breast cancer is recognized as being heterogeneous in terms of its biological and clinical behaviour. Historically breast cancers have been classified by histological grade. The most common subtypes are reported as invasive ductal carcinoma not otherwise specified (IDC NOS), with 75% prevalence and invasive lobular carcinoma (ILC) with 10% prevalence [1]. With the development of rapid immunohistological screening techniques, classification by receptor status has also become common. Tumour profiling by the presence or absence of

the oestrogen (ER), progesterone (PR) and human epidermal growth factor receptor 2 (HER2) are now routinely performed. Unfortunately, receptor status correlates poorly with histological grading and so both techniques are widely utilized to guide clinical decision making [2]. In general, ER+ tumours respond best to available therapeutics and have the highest survival rates whereas triple negative cancers, lacking ER, PR and HER2, remain resistant to current treatment options and have the worst prognosis [3]. Further developments in experimental techniques allow researchers to type cancers based on DNA, RNA and microRNA markers but many of these classifications are prohibitively expensive and so are not immediately relevant to the clinical setting [1,4-7].

Triple negative breast cancers

Triple negative breast cancers (TNBCs) account for approximately 10-17% of all breast cancers [8]. Clinically, they are more prevalent in young women and there is some evidence of increased prevalence in African-American women [3]. TNBCs are poorly differentiated, highly malignant, more aggressive and have a poor outcome [9]. They are also characterized by high rate of early recurrence and visceral metastasis [10]. The molecular changes associated with TNBCs include *p53* mutation, overexpression of Ki67 and EGFR, and dysfunction in the BRCA1 pathway [11]. It is estimated that EGFR is expressed in 60% of TNBCs [12]. In addition, TNBCs have an over expression of

Table 1. Therapeutic targets and investigational agents for triple-negative breast cancer [14-16].

Target mechanism	Existing Therapeutic Options
Targeting aberrant DNA repair	PARP inhibitors DNA transcription inhibitors Platinum agents
Inhibition of microtubule function	Taxanes
Inducing release of Reactive Oxygen Species (ROS)	Doxorubicin Cisplatin
Anti-angiogenic agents	Bevacizumab Sunitinib
EGFR targeting	Cetuximab Erlotinib
Epigenetic modifications	Trichostatin A Butyrate Vorinostat
c-Kit targeted	Imatinib
Src inhibitor	Dasatinib
mTOR inhibitor	Everolimus

Table 2. Molecular targets of anticancer activity of curcumin in cancer cells [18].

Pathway	Target
Cytokines	<i>Decreased:</i> IL-1; IL-2; IL-5; IL-6; IL-8; IL-12; IL18; MCP; MIP; MaIP; TNF α
Growth Factors and receptors	<i>Decreased:</i> VEGF*; β -FGF*; HER; CTGF; FGF; HGF; TF; NGF; EGF; PDGF; TGF- β 1 <u>Receptors:</u> <i>Decreased:</i> ER- α ; HER-2; AR; EGRF; Fas-R; IR; EPCR; H2R; CXCR4; IL-8R; LDLR; ITR; AHR <i>Increased:</i> DR-5
Metastasis, angiogenesis and invasion	<i>Decreased:</i> MMP-2*; MMP-3*; MMP-9*; RON-TKR*; Integrin α (6) β (4)*; LAR*; CXCL1, 2*; ICAM-1; ELAM-1; IAP-1, VCAM-1; uPA <i>Increased:</i> TIMP-1,2*
Apoptosis molecules	<i>Decreased:</i> Bcl-2; Bcl-XL* <i>Increased:</i> Bax*; Bad*; P21*; P27*; mapsin*; P53; Caspase activation*; Depolymerization of mitotic microtubules*
Cell cycle proteins	<i>Decreased:</i> Cyclin D1; GSK3 b; E-Cadherin*; Cyclin E
Transcription factors	<i>Decreased:</i> NF κ B*; HIF-1*; Notch-1; EGR-1; WT-1; β -catenin*; AP-1; STAT-1; STAT-3; STAT-4; STAT-5 CREB-BP <i>Increased:</i> Nrf-2; PPAR- γ ; ERE
Enzymes	<i>Decreased:</i> COX-2*; GST; DNA pol; Telomerase; TMMP-3; Src-2; FPT; ODC; PhP-D; iNOS; NQO-1; 5-LOX; GICL; ATPase; ATFase; GCL; AATF-1 <u>Kinases:</u> <i>Decreased:</i> MAPK; ERFR-K; ERK; JAK; PAK; PKA; PKB; PTK; PhK; IL-1R AK; Pp60c-tk; Ca ²⁺ -PK; FAK; AAPP <i>Increased:</i> JNK
Other	<i>Decreased:</i> MDRP; HSP-70

*Indicates effect documented in TNBC cells, [19].

cytokeratins 5, 6, 14, and 17, smooth muscle actin, P-cadherin and c-kit [13,14]. These molecular alterations have led to intensive research efforts to identify drug entities that will specifically target TNBC cancers. A summary of some of the possible therapies is included in Table 1 [14-17].

One promising area of research for cancer chemotherapies, especially for drug-resistant cells, is the plant phenols. A wide range of compounds are currently under development with several showing promise as they target multiple pathways to effect cellular death. One chemical within this class which is attracting increased research attention is curcumin.

Curcumin as a chemotherapeutic

Curcumin, obtained from the roots and rhizomes of the perennial plant *Curcuma longa*, is cytotoxic towards both ER+ and TNBC cells [18-20]. For example, curcumin inhibited the proliferation of ER-MDA-MB-468 and MDA-MB-231 cells, with IC₅₀ values of 1 and 7.6 μ M, respectively [19,21]. Results from Lai et al., (2012) suggest that curcumin preferentially targets cell lines lacking ER with SKBr3 and MDA-MB-231 (both ER-) cells being more sensitive to curcumin exposure than the ER+ lines BT474 or MCF7 [19,20]. Curcumin's mechanism of action appears to be multifaceted. Research has reported effects on inflammatory cytokines, growth factor receptors, metastatic and invasion markers, apoptosis related proteins and cell cycle proteins. A summary of these actions is provided in Table 2 [22,23].

Curcumin induces apoptosis in most, if not all, breast cancer cell lines and this occurs via activation of the mitochondrial-dependent apoptotic pathway [24]. In most cells, curcumin induces a loss of the mitochondrial membrane potential, opening of the transition pore, releasing cytochrome c, activating caspase-9 and caspase-3 and cleaving PARP, ultimately leading to DNA fragmentation and apoptosis [25] [26]. Down regulation of anti-apoptotic proteins (Bcl-2 and Bcl-XL) and up regulation of pro-apoptotic proteins (Bad and Bax) also leads to curcumin-induced apoptosis in many cancer cells including breast cancer [25]. Interestingly, many of these effects appear to be independent of the receptor status of the cell, with triple negative cell lines being similarly or more susceptible to curcumin than cell lines where the ER, PR or HER2 is present [27,11].

Curcumin is also a potent inhibitor of angiogenesis inhibiting both VEGF and β -FGF expression in MDA-MB-231 cells at 50 μ M. Low doses of curcumin(15 μ M) inhibited the invasive potential of MDA-MB-231 cells by down regulation of MMP-2, MMP-3 and MMP-9 and up regulation of tissue inhibitor metalloproteinase (TIMP-1, 2) [27,28]. Curcumin(from 10 μ M) inhibited integrin α (6) β (4), a laminin adhesion receptor in MDA-MB-231 cells and thus inhibited cell motility and invasion [29]. Similarly, in an *in vivo* xenograft model, the administration of curcumin (2% w/w in the diet) significantly decreased the incidence of breast cancer metastasisto the lung at five weeks. This was associated with suppression of the expression of NF κ B, COX2

and MMP-9 [30]. This finding was corroborated by a similar study where a lower dose (1% w/w in the diet) suppressed lung metastasis of breast cancer tumours after 21 days. Results showed that treatment with curcumin was associated with a decrease in NF κ B, AP-1 and MMP-9 expression levels [31].

Improving naturally derived compounds – combination studies

The use of curcumin as a clinical drug is limited by poor bioavailability. Many groups have investigated whether combining curcumin with other existing or promising therapies may alleviate this problem. For example, a combination of the two naturally derived compounds curcumin and epigallocatechin gallate (EGCG) was effective in both *in vitro* and *in vivo* models of TNBC. In MDA-MB-231 cells these two compounds (EGCG at 25 μ M and curcumin at 3 μ M) increased apoptotic cells and G2 arrest 2.6-fold compared to curcumin alone [32]. This effect was only observed in TNBC cells and not in MCF-7 cells. Importantly, this *in vitro* effect translated to tumour suppression *in vivo*. Specifically, curcumin (200 mg/kg) and EGCG (25 mg/kg) significantly suppressed MDA-MB-231 xenograft tumour volume by 49% compared to vehicle control after 10 weeks of treatment [32]. This was in part driven by a 78% decrease in VEGFR-1 protein expression in tumours.

Curcumin has also been reported to increase the sensitivity of various cancer cell lines to existing therapies such as doxorubicin [33], tamoxifen [34], cisplatin [33], camptothecin, daunorubicin, vincristine and melphalin [35]. Conversely, curcumin has also been reported to decrease the response of cells to camptothecin, doxorubicin and mechlorethamine [36]. The difference in results appears to be dose related with the latter study being conducted to mimic a dietary intake of curcumin (less than 1 μ M) with the other studies testing a therapeutic scenario with curcumin concentrations at 10 μ M or greater.

In breast cancer cells the combination of curcumin with paclitaxel in an MDA-MB-231 xenograft model, caused a significant reduction in tumour growth. Interestingly, the dose of paclitaxel was much lower (7 mg/kg) than previously reported as a single treatment [37]. Success has also been shown using *in vitro* combination studies. Specifically, when curcumin was combined with piperine the two drugs worked synergistically to inhibit breast cancer stem cell self-renewal without affecting normal cells [38]. Synergistic growth inhibition and the induction of apoptosis in MDA-MB-231 cells also occurred following the combination of curcumin and xanthorrhizol [39].

A recent phase II trial was designed to test the maximal tolerated dose of curcumin when co-administered in combination with docetaxel. While details were not provided, the researchers concluded that on-going investigation of the combination treatment was warranted suggesting some clinical gains over docetaxel administration alone [40]. The results from this study also demonstrated that the maximal

tolerated dose of curcumin was 6000mg/day for seven days when given in combination with a standard docetaxel regime (1 h i.v. infusion every 3 w on d 1 for six cycles) [40].

Novel drug delivery of natural compounds

An alternative to combination therapy regimes is to utilize emerging nanotechnology-based drug delivery systems. Various structures such as nanoparticles, liposomes, micelles, adjuvants and phospholipid complexes have been developed in order to specifically target cancer cells in order to improve efficacy and bioavailability, while reducing toxicity [41]. Nanoparticles can improve the bio-distribution of drugs, as they are able to act as carriers of anti-cancer drugs by selectively using the unique pathophysiology of tumours, such as their enhanced vascular permeability and extensive angiogenesis. This improved efficacy can be demonstrated with gelatine nanoparticles which show increased accumulation within the tumour and prolonged *in vivo* circulation [42].

Curcumin loaded silk-fibroin nanoparticles (< 100 nm) exhibited higher uptake, intracellular residence time and efficacy against HER2 positive MDA-MB-453 breast cancer cells [43]. Curcumin-oligo ethylene glycol (Cur-OEG) nanoparticles have also been studied for their anticancer effect in both *in vitro* and *in vivo* models of breast cancer. Curc-OEG nanoparticles showed *in vitro* antitumor activity towards several human cancer cells with an IC₅₀ value of 1.4 μ g/ml in MDA-MB-468 cells. Furthermore, intravenous injection of Curc-OEG nanoparticles (25mg/kg) was non-toxic to the mice and exhibited improved bioavailability and significant tumour suppression after 48 h in an MDA-MB-468 xenograft model.

In an alternative system, curcumin was encapsulated in poly(lactic-co-glycolide) (PLGA). These loaded nanoparticles elicited a six-fold increase in curcumin uptake and increased cytotoxicity in MDA-MB-231 cells as compared to curcumin alone [44]. *In vivo*, a single dose of subcutaneously injected PLGA-particles sustained curcumin levels in the blood for 30 days with 1- to 30 fold increased curcumin concentrations detected in the lungs and brain compared to the blood [45]. Furthermore, curcumin (58.2 mg/mouse, injected 24h prior to tumour inoculation) inhibited the growth of tumours in MDA-MB-231 xenografts by 49% compared to the mice treated with blank PLGA-particles or native curcumin (4.4mg,i.p.) [45]. The mechanisms of tumour reduction mirrored *in vitro* results with decreases in VEGF (decreased 78% vs. control), MMP-9 (57%), Ki67 (45%) and Cyclin-D1 (52%). Interestingly, repeated systemic dosing of curcumin had no effect on tumour growth or apoptosis markers. Therefore, the study concluded that sustained release microparticles of curcumin are more effective than repeated systemic injections of curcumin for breast cancer chemoprevention. A second *in vivo* study showed complementary results with curcumin nanoparticle formulation having a higher bioavailability and longer half-life in rats compared to free curcumin. Specifically, after intravenous administration of curcumin-nanoparticle (2.5

mg/kg), the serum levels of curcumin after 24 h were more than double those in the non-nanoparticle treated rats [46]. *In vitro* results from this study also showed decreased protein levels of VEGF, MMP-9 and Cyclin-D1.

Multi-functional nanoparticles are also now under development. These layered systems are designed to maximize drug delivery; allow MRI, magnetic, or thermo-targeted activation; and enhance imaging capabilities [44]. Curcumin loaded magnetic nanoparticles had greater cellular uptake as compared to PGLA-based formulations and in MDA-MB-231 the observed IC_{50} values of curcumin decreased from 18.8 to 11 μ M. This was in spite of the observation that due to the sustained release of curcumin from the magnetic nanoparticle only 40% of the loaded drug had been released within the measurement timeframe. The authors suggest that the overall *in vivo* tumour suppressive effect would be significantly greater [44].

Synthetic derivatives

An alternative to encapsulation systems to enhance the bioavailability of curcumin is chemical alteration of the base structure to increase systemic uptake. The aim of these modifications is to produce a compound with greater stability, bioavailability and ultimately efficacy. The basic structure of curcumin is of two phenol groups connected by two α,β -unsaturated carbonyl groups (Table 3). The central diketones can form stable enols which are readily deprotonated to enolates. The equilibrium between the ketone, enol and enolate forms is responsible for the antioxidant properties of curcumin [47]. Modification of the phenolic rings and β -diketone moiety of curcumin has led to a range of different curcumin analogues with improved activities (Table 3) [48]. The first-generation curcumin derivatives exhibited enhanced activity and stability in biological medium compared to curcumin.

The curcumin analogues (FLLL 11 and FLLL 12) produced by exchanging the β -diketone moiety for an α,β unsaturated ketone, exhibited more potent antitumor activity than curcumin in various ER+ and ER- human breast cancer cells [49]. The IC_{50} values for FLLL11 and FLLL12 ranged from 0.3 to 5.7 μ M, significantly lower than curcumin in equivalent cell lines. Both the analogues inhibited STAT3, Akt and HER2/Neu pathways and induced apoptosis at concentrations of 10 μ M. The apoptosis was mediated via activation of cleaved PARP and caspase 3. These analogues were also effective in combination with doxorubicin as they exhibited a synergistic anti-proliferative effect in MDA-MB-231 breast cancer cells. In addition, the compounds inhibited anchorage independent growth and cell migration in MDA-MB-231 cells [49].

The evidence from FLLL11 and FLLL12 suggests that incorporating one or more pairs of methoxy groups is key to increasing cytotoxicity. Otori et al., (2006), screened over 50 curcumin derivatives [50]. The most potent were GOYO35 and GOYO30, which incorporated multiple methoxy groups

at either end of the compound. In MDA-MB-231 cells GOYO30 has a reported IC_{50} of 1.2 μ M [51], significantly less than curcumin. Compound 15H with a combination of methoxy additions and pentone ring incorporation showed an IC_{50} of 1.9 μ M in MCF7 cells, the lowest reported value in these ER+ cells to date [52]. Fuchs et al., (2009), conducted a screen of twenty four compounds. Of those analysed GOYO21, with three methoxy pairs, was the most potent. GOYO21 (also reported as compound 23) showed toxicity at sub-micromolar concentrations in MDA-MB-231 cells (0.6 μ M) [53]. The major screening study conducted by Otori et al., (2006), identified three key features of potent cytotoxic derivatives: 1) a 5-carbon vs. 7-carbon tether in the central portion of the molecule; 2) the importance of the methoxy residues; and 3) maintenance of the enone/ketone moiety [50]. However, the importance of the enone group is disputed. Previously, Ishida et al., found that converting the enone to a pyrazole group resulted in the most potent compound in their screen of thirty two curcumin derivatives. The resulting chemical, labelled compound 4 was only tested in MCF-7 cells where it had a reported IC_{50} value of 3.9 μ M [54].

Second generation curcumin derivatives have focused on the importance of the central structure and are based on the inclusion of a heterocyclic or piperidine ring in place of the ketone moiety. This imparts a rigid confirmation that has led to a broad spectrum of antitumor activity. The second generation curcumin derivative 2,6-bis ((3-methoxy-4-hydroxyphenyl) methylene)-cyclohexanone (BMHPC) was cytotoxic toward ER- breast cancer cells (IC_{50} of 5.0 μ M) and displayed anti-angiogenic properties in human and murine endothelial cell lines [18,21,49,50,52-59].

Two further analogues, 2,6-bis(pyridin-3-ylmethylene)-cyclohexanone (RL90) and 2,6-bis(pyridin-4-ylmethylene)-cyclohexanone (RL91) (Table 3) also showed increased cytotoxicity towards ER- breast cancer cells (MDA-MB-231, SKBr3). Interestingly, removing the phenolic side groups and replacing them with piperidine rings increased cytotoxicity towards breast cancer cells markedly (IC_{50} of 0.51 and 0.23 μ M, respectively) [56]. Both RL90 and RL91 modulated the expression of variety of cell signalling proteins such as EGFR, Akt, HER2, β -catenin and NF κ B [56]. Treatment with RL90 and RL91 also showed activation of stress kinases, as evidenced by phosphorylation of both JNK1/2 and p38 MAPK. Furthermore, RL90 and RL91 produced cell cycle arrest at G2/M phase in MDA-MB-231 and SKBr3 cells. Specifically, treatment of MDA-MB-231 cells with RL90 (3 μ M) or RL91 (2.5 μ M) significantly increased the proportion of cells in G2/M phase by 52 and 49% compared to control, respectively. RL90 and RL91 also increased the proportion of apoptotic cells by 164% and 406% of control, respectively [56].

Compound, 8 and 18 (Table 3) bearing the *n*-alkyl piperidone group showed potent cytotoxic activity towards various breast cancer cells [57]. This structure was recently further modified by replacing the methylene groups and the two

Table 3. Chemical structures of curcumin derivatives and their relative *in vitro* potency. (NR* - Not reported)

Compound Name	Structure	Cell line	IC 50(μM)	Ref
Curcumin		MDA-MB-231 MDA-MB-468	7.6 1	16a 17
First Generation Derivatives				
FLLL11		MCF-7 MDA-MB-231 MDA-MB-468 MDA-MB-453 SKBr3	2.4 2.8 0.3 4.7 5.7	44
FLLL12		MCF-7 MDA-MB-231 MDA-MB-468 MDA-MB-453 SKBr3	1.7 2.7 0.3 1.3 3.8	44
GO-YO30		MDA-MB-231	1.2	45
15H		MCF7	1.9	47
Compound 23 (GOYO21)		MCF-7 MDA-MB-231	2.4 0.6	48
Compound 4		MCF-7	3.9	49
Second Generation Derivatives				
BMHPC		MDA-MB-231	5 2.6	50 15a
RL90		MDA-MB-231 SKBr3	1.54 0.51	51
RL91		MDA-MB-231 SKBr3	1.10 0.23	51
Compound 18		MCF-7 MDA-MB-31 MDA-MB-435 HS-578T BT-549 T-47D	3.3 2.8 3.7 3.8 2.6 2.7	52

Continuation of **Table 3**.

Compound Name	Structure	Cell line	IC ₅₀ (μ M)	Ref
Compound 8		MCF-7	2.3	52
		MDA-MB-31	17.9	
		MDA-MB-435	6.8	
		HS-578T	5.4	
		BT-549	32.8	
		T-47D	15.1	
PAC		BEC114	NR*	53
		MDA-MB-231	NR*	
		MCF7	NR*	
		T47D	NR*	
EF-24		MDA-MB-231	0.8	54
RL66		MDA-MB-231	0.8	15a
		MDA-MB-468	0.5	
		SKBr3	0.6	
RL71		MDA-MB-231	0.3	15a
MDA-MB-468	0.3			
SKBr3	0.4			

carbonyl groups in curcumin by N-methyl-4 piperidone. The resulting compound 5-bis (4-hydroxy-3-methoxybenzylidene)-N-methyl-4-piperidone (PAC) (**Table 3**) was 5 times more effective than curcumin in inducing apoptosis in ER- breast cancer cells (MDA-MB-231, BEC114) [58]. Additionally, the proapoptotic effect was 10 times higher against these ER- breast cancer cells than against ER+ cells (MCF-7, T-47D). *In vivo*, PAC (100 mg/kg/day) suppressed the growth of MDA-MB-231 xenografts [58]. Importantly, the solubility of PAC was 27-fold higher than curcumin and 1 h after the injection, the levels of ¹⁸F-PAC in the blood was 5-fold higher than the levels ¹⁸F-curcumin. These studies suggested better pharmacokinetics and tissue bio-distribution of PAC compared to curcumin in mice [58]. Cell cycle analysis revealed that PAC (10 μ M) treatment of MDA-MB-231 cells increased the proportion of cells undergoing G2/M phase arrest and induced apoptosis in 55% of MDA-MB-231 cells [58]. PAC down regulated the expression of NF κ B, surviving and its downstream effectors cyclin D1 and Bcl-2 and subsequently showed up-regulation of p21^{WAF1} expression both *in vitro* and *in vivo*.

Several fluorinated derivatives have also been synthesized. Of these, EF-24 exhibited potent cytotoxicity toward MDA-MB-231 cells (IC₅₀ of 0.8 μ M) [59] and induced breast tumour

regression in athymic nude mice. Specifically, tumour weight following 20 mg/kg was decreased by approximately 30% compared to control, whereas 100 mg/kg decreased tumour weight by 55%. Interestingly, no toxicity was observed at a dose of 100 mg/kg, which was well below the maximum tolerated dose (MTD) of 200 mg/kg [59]. Mechanistic studies of EF-24 were also performed in MDA-MB-231 cells. Results show cell cycle arrest in the G2/M phase and increased intracellular ROS levels after 48 hours [59]. Further mechanistic studies demonstrated that EF-24 inhibited the pro-angiogenic transcription factor, HIF-1 α at the post-transcriptional level by a VHL-dependent but proteasome-independent mechanism in MDA-MB-231 cells [60]. Using coagulation factor VIIa (fVIIa) as a carrier to target delivery to the tissue factor (TF), EF-24 significantly decreased the viability of the TF-expressing MDA-MB-231 and HUVEC cells in a concentration dependent manner. Furthermore, the administration of 5 intravenous injections of the EF-24-FFRck-fVIIa conjugate (containing 50 μ M of EF-24) for two weeks significantly reduced the tumour size in MDA-MB-231 breast cancer xenografts. The tumour cells also showed activation of caspase 3, a marker of apoptosis [61]. This suggests that it has potent anti-angiogenic and anti-cancer activity in breast cancer cells.

Yadav et al., (2010) examined 18 second generation curcumin analogues. Of these two compounds showed potent cytotoxicity: 3,5-bis (pyridine-4-yl)-1-methylpiperidin-4-one (RL66) and; 3,5-bis (3,4,5-trimethoxybenzylidene)-1-methylpiperidin-4-one (RL71) (**Table 3**). Both had IC₅₀ values below 1 mM in oestrogen receptor negative cell lines (MBA-MB-231 and MDA-MB-468) [18]. RL66 at 2 μM caused cell cycle arrest, a decrease in HER-2/neu phosphorylation, increased p27 levels, caspase 3 cleavage and apoptosis in SKBr3 cells after 48 h [62]. Furthermore, in MDA-MB468 cells RL66 (2 μM) decreased Akt phosphorylation and transiently increased JNK1/2 and MAPK p38 phosphorylation. Cell migration in HUVEC cells was inhibited by 46% indicating that RL66 is anti-angiogenic [62]. *In vivo*, RL66 (8.5mg/kg orally) decreased the growth of MDA-MB-468 cell xenografts over a 10 week treatment period [62]. Additionally, microvessel density in the tumors was decreased by 57% as compared to control. Based on these results the authors generated a structure with the central piperidine ring and added the methoxy groups identified from the stage one investigations. The resulting compound, RL71, is the most potent curcumin derivative developed to date. *In vitro* results show that it has consistent cytotoxicity across breast (ER-) [18], (ER+ and tamoxifen-resistant) (Leung personal communication), prostate (Mazumder personal communication), colon [63] and leukemia cell lines [18]. Similar to RL66 and the parent compound curcumin it has a broad mechanism of action including inhibition of EGFR, Akt, NFκB, mTOR and Her-2⁶¹. RL71 has also shown potent efficacy in a range of migration and invasion models of metastasis. Specifically, 1 μM of RL71 significantly inhibited HUVEC cell migration by 46% compared to vehicle control and completely inhibited endothelial tube formation [64]. Furthermore, in MDA-MB-231 cells 1 μM RL71 significantly reduced cell migration into a scratch area after 24 h [64].

Overall, curcumin is an exciting potential chemotherapeutic. While the parent compound has been shown to have cancer-specific toxicity and a range of target mechanisms, its use is limited by poor bioavailability. However, a range of new technologies means that this drug may be able to be adapted for clinical use. Specifically, by using encapsulation techniques such as micelles, targeted delivery can be achieved. These systems show a great deal of promise in pre-clinical studies with curcumin micelles being highly cytotoxic in a range of *in vivo* assays. Alternatively, the synthesis of derivatives such as RL71 demonstrates that the cytotoxicity of curcumin can be retained, or even improved, while increasing the absorption across the gut lumen. Mechanistic studies show that the mechanism of action for curcumin and the derivatives is broad meaning that they show potential in currently untreatable cancers. For patients with triple negative breast cancer, where current therapies show little efficacy and the need for new therapies is imperative, these potent derivatives have the potential to form the basis for a new effective treatment.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Authors' contributions	BJC	LL	RJR
Research concept and design	--	√	√
Collection and/or assembly of data	√	--	--
Data analysis and interpretation	√	--	--
Writing the article	√	--	--
Critical revision of the article	--	--	√
Final approval of article	--	√	√
Statistical analysis	--	--	--

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