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The anti-cancer effects and mechanisms of lactic acid bacteria exopolysaccharides *in vitro*: A review

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ABSTRACT

Probiotic lactic acid bacteria (LAB) are a particular group of gram-positive bacteria that are usually involved in natural ferments and widely used in food manufacture industry. Most of them can produce exopolysaccharides (EPS), surface carbohydrate polymers with diverse biological functions. LAB EPS are potentially complementary and alternative medicines against cancer. EPS show anti-proliferative effects on a variety of tumor cells from intestine, liver, breast, *etc.* They modulate the development of tumors through various mechanisms including promoting apoptosis, inducing cell cycle arrest as well as anti-mutagenic, anti-oxidative, anti-angiogenesis and anti-inflammatory effects. Bacterial origin, existence form, chemical structure, purity et al. are important factors affecting the anticancer effects of EPS. The future challenge lies in elucidating the precise structure-function relationship of LAB EPS. Besides, more *in vivo* studies and further clinical trials are indispensable to confirm the anticancer effects.

1. Introduction

Cancer refers to the rapid replication of abnormal cells with the potential to overgrow, invade the adjacent tissue and metastasize to other organs. Data from the Global Cancer Observatory showed that cancer had become the second major cause of death worldwide and lead to approximately 9.6 million deaths in 2018 (Bray et al., 2018). Current cancer managements (surgery, irradiation and chemotherapy) exist many undesirable side and adverse effects. Besides, it is usually unaffordable and unavailable to cure cancer in some places. As a result, complementary and alternative medicines are preferred by people for its affordability, availability and lower side effects (Erejuwa, Sulaiman, & Wahab, 2014).

Some foods and food components can improve malnutrition, reduce the incidence of certain types of cancer as well as decrease the adverse effects of chemotherapy, thus are considered as important adjuvants for cancer therapy. One of them is the fermented milk product, which is confirmed to be protective against colorectal cancer (Rafter, 2004; Saikali, Picard, Freitas, & Holt, 2004). Fermented milk product is a kind of dairy food fermented by lactic acid bacteria (LAB), probiotics that exist in human intestine with various beneficial effects on the host's health. Intranasally administered *Lactobacillus casei (L.casei)* BL23 in the allograft model of HPV-induced cancer showed significant protective effects against tumor onset (Jacouton et al., 2019). *In vitro* study showed

Abbreviations: 4-NQO, 4-nitroquinoline N-oxide; ABTS, 22′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AP-1, activating protein-1; BCL2, B-cell lymphoma 2; BrdU, 5-bromo-2-deoxyuridine; cb-EPS, cell bound EPS; CCK8, cell counting kit-8; COX2, cyclooxygenase 2; CFU, colony-forming units; CYCS, cytochrome c; DPPH, 22-diphenyl-1-picrylhydrazyl; EAC, Ehrlich Ascites Carcinoma; EPS, exopolysaccharides; FADD, FAS-associated death domain; FASL, FAS ligand; HePS, heteropolysaccharides; HIF, hypoxia-inducible factor; HoPS, homopolysaccharides; IC_{50}, 50 % proliferation inhibition of tumor cells; IL, interleukin; iNOS, inducible nitric oxide synthase; IPEC-J2, porcine small intestinal epithelial cell line; LAB, lactic acid bacteria; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MOMP, mitochondrial outer membrane permeabilization; MTT, 3-45-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Mw, molecular weight; NF-κb, nuclear factor kappa B; NO, nitric oxide; PARP, poly ADP-ribose polymerase; PBMC, peripheral blood mononuclear cell; r-EPS, released EPS; ROCK1, Rho-associated coiled-coil containing protein kinase 1; ROS, reactive oxygen species; SRB, sulforhodamine B; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor β; TIMP3, tissue inhibitor metalloproteinases 3; TLRs, toll-like receptors; TNF, tumor necrosis factor; TNFRS/DRS, TNF receptors; TRADD, TNF receptor-associated death domain; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

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that live cells of *Lactobacillus reuteri (L.reuteri)* BCRC14652 (10^8 CFU/mL or 10^9 CFU/mL) induced cell membrane damages to human colon carcinoma cells HT29 (Chen, Hsieh, Huang, & Tsai, 2017). Another study found live *Lactobacillus acidophilus (L.acidophilus)* and *L.casei* mix (10^7-10^9 CFU/mL) can dose-dependently increase the apoptosis-induction capacity of 5-fluorouracil, a conventional cancer drug (Baldwin et al., 2010). Interestingly, studies also confirmed the anti-tumor potentials of the cultured supernatants and the dead cells (such as irradiation-inactivated cells and heat-killed cells) of LAB (Baldwin et al., 2010; Chen et al., 2017; Choi et al., 2006; Hsieh et al., 2016; Tuo et al., 2015). This arouses the curiosity of researchers to find out the precise component inside or outside LAB that play a major role in anti-tumor effects. 4-nitroquinoline N-oxide (4-NQO) is a chemical pro-mutagen that is able to induce DNA damage, and extracellular polysaccharides (EPS) were found to be the most effective in reducing its cytotoxicity, compared with other bacteria fractions including intracellular extracts, crude cell walls that obtained from the same amount of *L. casei* 01 (Liu, Chu, Chou, & Yu, 2011). Another study comparing the anti-proliferative effects of EPS, extracellular protein and lipid of the same concentration (0.1 mg or 10 mg/mL) also found that the activity of EPS isolated from *L. acidophilus* 606 was the most remarkable on various colon cancer cell lines while the other two had no anti-proliferative effects (Choi et al., 2006). Further *in vivo* animal-based studies confirmed the anti-cancer efficacy of EPS by intraperitoneally injecting or intragastrically administering EPS into tumor-bearing mice (Abd El Ghany et al., 2014, 2015; Adebayo-Tayo & Fashogbon, 2020; Haroun, Refaat, El-Menoufy, Amin, & El-Waseif, 2013; Zahran, Elsonbaty, & Moawed, 2017). The results showed that EPS significantly increased the lifespan of mice with cancer for about 60–80 % (Adebayo-Tayo & Fashogbon, 2020; Haroun et al., 2013). Tumor volume was significantly reduced by EPS from *Lactobacillus plantarum (L.plantarum)* and *L. acidophilus*, reflected directly by measuring the length, width and height of the tumor or indirectly by measuring the body weights of the mice (Abd El Ghany et al., 2014, 2015; Haroun et al., 2013).

As the anti-tumor effects of LAB EPS have attracted broad attention, this review highlights the anti-tumor effects and mechanisms of LAB EPS observed *in vitro*. Pubmed and EMBASE were searched for studies up to September, 2020 using anti-cancer, lactic acid bacteria and exopolysaccharide as the key words. Additional researches were obtained by manually searching the references from relevant articles. One thing should be pointed out that the genera of LAB included in this review were *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus,* and *Weissella*, which played an important role in industrial manufacture (de Souza de Azevedo et al., 2020). Flow chart of inclusion and exclusion process was demonstrated in Fig. 1. Since the food industries favor EPS as natural biothickeners, which provide products with suitable consistencies, improved viscosity and reduced syneresis (Ruas-Madiedo & de los Reyes-Gavilán, 2005), it is available to intake EPS orally in daily life. Thus, it is worthy of reviewing the anti-cancer mechanisms of LAB EPS in order to provide some innovative ideas for cancer prevention and treatment.

2. Anti-cancer effects and mechanisms

2.1. Apoptosis induction

After treated with EPS, tumor cells show characteristic apoptotic morphological changes including vacuolation, cytoplasm condensation, nuclear disintegration, and chromatin condensation. Besides, apoptotic bodies surrounding the nucleus and swollen mitochondria can also be observed (Deepak, Ramachandran et al., 2016, 2018; Di et al., 2017; Sun et al., 2018; Wei, Li, Li, Huang, & Li, 2019; Wu et al., 2016). Compared with a thick and continuous out layer in the untreated tumor cells, cell membranes of HCT15 and Caco2 became rough and discontinuous after treated with 5 mg/mL EPS from *L. acidophilus* 10307 for 48 h (Deepak,

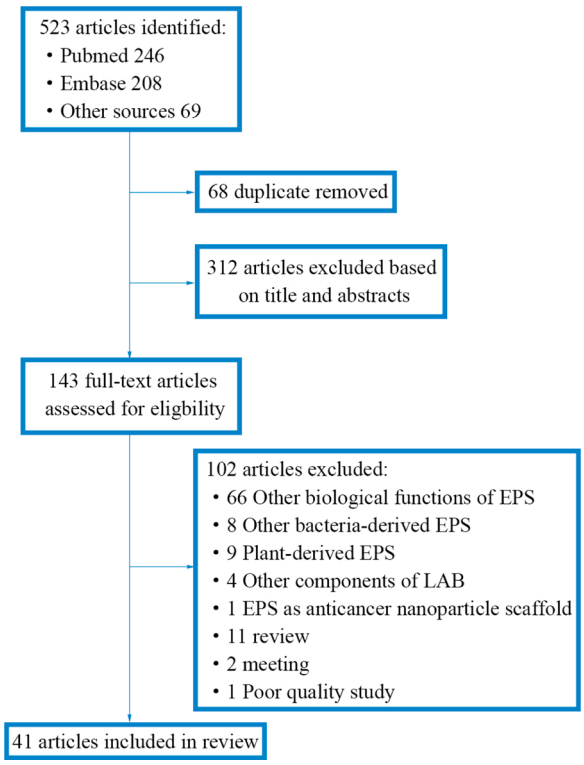

Fig. 1. Inclusion and exclusion processes.

Ramachandran et al., 2016). The dose-dependently enhanced lactate dehydrogenase (LDH) leakage in the cell culture media also reflects the membrane damage effects of EPS (Deepak, Ramachandran et al., 2016; Kim, Oh, Yun, Oh, & Kim, 2010).

At the center mechanism of apoptosis stands cysteine aspartyl-specific proteases (caspases). Caspase-2, -8, -9, -10 belong to initiator caspases and caspase-3, -6, -7 are executioner caspases. The extrinsic or death receptor pathway, and the intrinsic or mitochondrial pathway are the two pathways that activate caspases and lead to apoptosis. In the extrinsic pathway, cell death signals (death ligand) including FAS ligand (FASL), tumor necrosis factor (TNF), and TNF-related apoptosis-inducing ligand (TRAIL) combine with death receptors such as FAS and TNF receptors (TNFRS/DRS). Then the adaptor proteins including the FAS-associated death domain (FADD) and TNF receptor-associated death domain (TRADD) are recruited. In this pathway, caspase-8 is activated, followed by caspase-3 (Pfeffer & Singh, 2018). EPS isolated from *L. plantarum* NCU116 (EPS116) specifically activates the transcription and translation of FAS and its ligand FASL *via* TLR2/MyD88/TRAF6/MKK7/JNK/c-Jun pathway (Zhou et al., 2017). The gene expression of tumor necrosis factor (TNF) receptors tested including *Tnfr1*, *Tnfr2*, *Dr3*, and *Dr5* demonstrate no significant variation. No obvious differences were observed in the expression levels of *Apaf1, cytochrome c (Cycs), Bcl2, and Bax* in CT26 (mouse epithelial colorectal cell line) after EPS116 treatment, and these four molecules are the key elements that function in the intrinsic pathway (Zhou et al., 2017). However, the intrinsic pathway still takes a role in the anti-tumor effects of other kinds of LAB EPS. B-cell lymphoma 2 (BCL2) protein family initiate the intrinsic apoptosis pathway. It can be divided into three groups: (1) anti-apoptotic proteins BCL2, BCL2L1, BCL2L2 et al. containing all four BH regions; (2) pro-apoptotic proteins BAD, BIM, BID, BECLIN1 et al. with only BH3 region, thus named BH3-only proteins; (3) pro-apoptotic proteins like BAX and BAK, which contain BH 1–3 regions (Shamas-Din, Brahmbhatt, Leber, & Andrews, 2011). The former two subsets regulate the expression of the latter one through antagonism. The activated BAX/BAK lead to mitochondrial outer membrane permeabilization (MOMP). The intermembrane proteins

such as CYCS are released which causes the formation of apoptosome. Within the apoptosome, caspase-9 is activated which further activates caspase-3 (Pfeffer & Singh, 2018). After treated with LAB EPS, tumor cells demonstrated reduced anti-apoptotic protein BCL2 (Kim et al., 2010; Tukenmez, Aktas, Aslim, & Yavuz, 2019) and increased BH3-only pro-apoptotic proteins such as *BAD* (at gene level) and BECLIN1 (Di et al., 2017; Kim et al., 2010). This change induces the production of BAX/BAK (Di et al., 2017; Kim et al., 2010; Sungur, Aslim, Karaaslan, & Aktas, 2017; Tukenmez et al., 2019). The potential of MOMP is enhanced (Wu et al., 2016). Though *APAF1* and *CYCS* seem not to be affected by EPS, the expression of caspase-9 still goes up (Tukenmez et al., 2019; Wu et al., 2016). EPS reduces the production of survivin (Tukenmez et al., 2019), a small protein belonging to the inhibitor of the apoptosis protein family (Martínez-García, Manero-Rupérez, Quesada, Korrodi-Gregório, & Soto-Cerrato, 2019). A few kinds of caspase-3 substrates including ACINUS, GAS2, LAMIN, Poly ADP-ribose polymerase 1 (PARP1), PARP2 and Rho-associated coiled-coil containing protein kinase 1 (ROCK1) have been measured. The cleavage of PARP1 and the production of ROCK1 are increased after EPS treatment (Zhou et al., 2017). PARP1 contributes to DNA repair and participates in many pathways of cell death. Its cleavage by caspase-3 has been identified as a biochemical marker of apoptosis (Oliver et al., 1998). ROCK1 cleaved by caspase-3 leads to myosin light chain phosphorylation, resulting in apoptosis initiation, cell shrinkage and membranes blebbing (Sebbagh et al., 2001). Factors that have been confirmed to be changed are marked with blue (reduced) or red (increased) in Fig. 2. Table 1 summarizes the bacterial source, existence form, effective dosage, and modified factors of EPS that can promote apoptosis of cancer cells reported so far. Not all the molecules tested change in the desired direction in different studies (key findings in Table 1), which might be because of the different apoptosis mechanisms of EPS derived from different LAB as well as the complexity of the apoptosis signal pathway.

The interaction among BCL2 protein family members forms the primary mechanism of the cross-talk between autophagy and apoptosis. Pro-apoptotic protein BECLIN1 is at the same time a component of the class III phosphatidylinositol 3-kinase complex, the activation of which is one of the initial steps of autophagy (Menon & Dhamija, 2018). The BECLIN1 BH3 domain combines directly with BH3-binding grooves on BCL2/BCL2L1, effectively suppressing autophagosome biogenesis (Menon & Dhamija, 2018). And their combination is separated by BH3-only proteins like BAD (Erlich et al., 2007). ROCK1 promotes autophagy by binding and phosphorylating BECLIN1 (Gurkar et al., 2013). PARP1 participates autophagy induced by DNA damage probably by its interplay with the mammalian target of rapamycin (Muñoz-Gámez et al., 2009). However, there is no direct evidence that EPS promotes cancer cell death through autophagy.

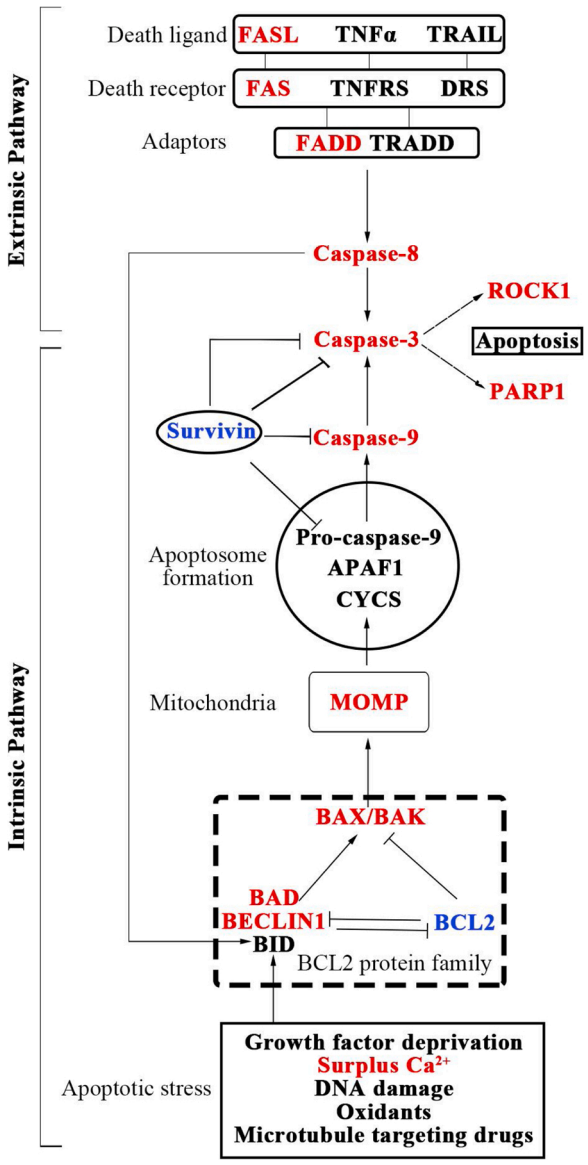

Fig. 2. Promotion of tumor cell apoptosis by LAB EPS. LAB EPS promote pro-apoptotic molecules (red font) and inhibit anti-apoptotic molecules (blue font) in the extrinsic and intrinsic apoptotic pathways (Pfeffer & Singh, 2018) of tumor cell lines (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.2. Anti-proliferation

EPS extracted from LAB showed anti-proliferative effects on various tumor cells in dose and time-dependent manner. The source of the most studied tumor cell line is the intestine, followed by the liver and breast. The rest include mammary, gastric, cervix, pancreas, and larynx (Table 2). The concentration causing 50 % proliferation inhibition of tumor cells (IC_{50}) by LAB EPS from different bacteria strains are summarized in Table 2. Since most studies did not calculate the exact IC_{50} values, the scopes that IC_{50} existed are shown instead. As Table 2 shows, most IC_{50} are around 1 mg/mL. The highest IC_{50} of EPS towards tumor cells is 34.7 mg/mL when EPS extracted from *L. plantarum* NRRL B-4496 acted on Larynx carcinoma cell lines HEP2 (Haroun et al., 2013). LW1, a high molecular weight fraction of exopolysaccharides purified from *L. casei* SB27, has the lowest IC_{50} between 0.01−0.05 mg/mL towards HT29 (Di et al., 2017). More researches studying the effects of the same EPS on different tumor cells confirm that EPS have different degrees of cytotoxic activities on different tumor cells. A more consistent conclusion is that EPS have a better inhibitory effect on intestine derived cancer cells, suggesting that EPS may be more suitable for the treatment of colorectal cancer (Ayyash, Abu-Jdayil, Itsaranuwat et al., 2020, b, Haroun et al., 2013; Li & Shah, 2016; Nguyen & Nguyen, 2014; Wang et al., 2014b; Wei, F., L., Huang, & Li, 2019). This may be because that EPS are more likely to bind to the surface receptors of intestinal cancer cells, since LAB are probiotics that widely exist in human intestine and contribute to colonic mucosal homeostasis. Further studies should be carried out to elucidate this point.

2.3. Selective cytotoxicity

Even though the IC_{50} for the LAB EPS is much higher than traditional anti-neoplastic drugs such as doxorubicin (Ismail & Nampoothiri, 2013) and fluorouracil (Li, Tang et al., 2015; Li, Xia et al., 2015, Wang et al., 2014a,b), the presence of very little cytotoxicity still make it an anti-tumor drug with wide application potential. The standard drug may be more toxic due to their inhibitory effects on DNA and RNA synthesis. *In vivo*, the lifespan of tumor induced mice treated with 500 nM aqueous

Table 1
Summary of pro-apoptotic attributes of LAB EPS.

LAB strain	Cancer Cell	Form	Structure			Effective apoptotic concentration	Key Findings	Ref.
		cb/r	HoPS/ HePS	monosaccharide composition	Mw (kDa)			
L. casei SB27	HT29	r	HePS	galactose, glucose	25.10/ 12.34	600 μg/mL	▪ Expression levels of ***BAD*(↑)**, ***BAX*(↑)**, ***caspase-3*(↑)** and ***caspase-8*(↑)** were increased.	(Di et al., 2017)
L. casei X12/K11/ M5/SB27	HT29	r	–	–	–	500 μg/mL	▪ Four kinds of tested EPS were able to active **caspase-3(↑)**.	(Di et al., 2018)
L. rhamnosus ATCC9595	PANC1/ HT29	r	HePS	galactose, glucose, rhamnose, mannose	8600/43	5 mg/mL	▪ The production of **caspase-3(↑)** was increased.	(Kim et al., 2006)
L. acidophilus 606	HT29	cb	–	–	–	10 mg/mL	▪ **BCL2(↓)** was reduced by 2.2-fold. ▪ **BAK(↑)** was induced by 1.5-fold. ▪ **BAX(-), PARP(-), AIF(-), and caspase-3(-)** had no significant variation. ▪ Autophagy-related proteins, **BECLIN1(↑)** and **GRP78(↑)**, were induced.	(Kim et al., 2010)
L. gasseri G10/ H15	Hela	r	HePS	Glucose, fructose, mannose, arabinose, maltose	–	400 μg/mL	▪ EPS of G10 significantly increased the expression of ***caspase-3*(↑)** and ***BAX*(↑)**. ▪ EPS was observed to have no impact on ***caspase-9*(-)** and ***BCL2*(-)**. ▪ The production of **TNFα(↓)** was significantly decreased by EPS of G10 and H15.	(Sungur et al., 2017)
L. plantarum GD2 *L. rhamnosus* E9 *L. brevis* LB63 *L. delbrueckii ssp. Bulgaricus* B3	HT29	r	HePS	Mannose, glucose	GD2: 2.4/0.23 E9: 10/ 0.27 LB63: 2.5/9.3/ 0.24 B3: 12/ 0.35	400 μg/mL	▪ The gene and protein expression demonstrated an upregulation of **BAX(↑)**, **caspase-3(↑)**, and **caspase-9(↑)** and a downregulation of **BCL2(↓)** and **survivin(↓)**.	(Tukenmez et al., 2019)
L. Lactis	MCF7	r	HePS	Ribose, fucose, mannose, glucose, galactose, arabinose	–	100, 200, 300 μg/mL	▪ The intracellular Ca^{2+}**(↑)** was induced. ▪ The production of **TNFα(↑)** was activated. ▪ Mitochondrial outer membrane permeabilization **(MOMP) (↓)** potential was reduced. ▪ The activated **caspase-9(↑)** resulted in the activation of downstream **caspase-3(↑)**.	(Wu et al., 2016)
L. plantarum NCU116	CT26	r	HePS	galactosamine, glucosamine, glucose, mannose, glucuronic acid	384	200, 400, 800 μg/mL	▪ First, EPS116 bound to TLR2 to activate TLR2/MyD88/TRAF6/MKK7 pathway. ▪ Then JNK/c-Jun was activated and the transcription and translation of **FAS(↑)** and **FASL(↑)** were upregulated. ▪ Fas-mediated apoptosis pathway activated **caspase-8(↑)** and **caspase-3(↑)**. ▪ Finally, activated caspase-3 increased **PARP1 cleavage (↑)** and **ROCK1 (↑)**. ▪ No significant variations were observed in intrinsic pathway factors (***Apaf1*(-)**, ***Bax*(-)**, ***Bcl2*(-)**, ***Cycs*(-)**), death receptors (***Apo2L*(-)**, ***Dr3*(-)**, ***Dr5*(-)**, ***Tnfr*(-)**), and caspase-3 substrates (***Acinus*(-)**, ***Gas2*(-)**, ***Lamin*(-)**).	(Zhou et al., 2017)

Note: ↑increase, ↓decrease, –no significant change after EPS treatment.
Abbreviation: cb, cell bound; HePS, heteropolysaccharides; HoPS, homopolysaccharides; Mw, molecular weight; r, released.

solution of EPS was much longer than the group treated with 20 mg/kg/day fluorouracil (Adebayo-Tayo & Fashogbon, 2020). Most EPS from LAB proved to have less (Choi et al., 2006; Haroun et al., 2013; Mojibi, Tafvizi, & Bikhof Torbati, 2019; Wei et al., 2019) or even no cytotoxicity (Das & Goyal, 2014; Liu et al., 2011) to normal cells than to the tumor cells. The *L. plantarum* EPS do not display cytotoxicity in normal fibroblast cells L929 until the concentration of EPS reaches 50 mg/mL (Ismail & Nampoothiri, 2013). The viability percentage of L929 after treated with 40 mg/mL EPS from *Lactobacillus paracasei (L. paracasei)* and *Lactobacillus brevis (L. brevis)* is higher than 60 %. Meanwhile, HT29 has less than 20 % survival rate (Mojibi et al., 2019). The selectivity index, which represents IC_{50} for normal cell line/IC_{50} for the cancerous cell line, ranges from 1.96 to 51.3 (El-Deeb, Yassin, Al-Madboly, & El-Hawiet, 2018). The highest selectivity index gets about 51.3, suggesting that the inhibitory effects of EPS on the proliferation of cancer cells might be due to the relative increment of the proliferation of non-cancerous cells (El-Deeb et al., 2018).

However, tumor cells are no more sensitive to EPS than some types of normal cells in some cases. EPS of *L. plantarum* NRRL B-4496 seem to have comparable inhibitory effects on HCT116 (colon carcinoma cell line) and HepG2 (liver carcinoma cell line) but fails to inhibit Hela (cervical carcinoma cell line) and HEP2 when comparing with normal melanocytes HFB4 (Haroun et al., 2013). 1 mg/mL EPS of *L. plantarum* RJF4 show cytotoxicity to neural glial cell line, but no cytotoxicity to DLD2 (colon carcinoma cell line) (Dilna et al., 2015). There is no uniform standard for the selection of normal cells in the control group. It might be of vital importance to compare the anti-tumor effects between tumor cells and normal cells from the same tissue. At a concentration of

Table 2
IC_{50} of EPS acting on cancer cell lines from different tissue sources.

Intestine										
cell	Species	Strain	Form	Structure			method	T/h	IC_{50}/mg•mL^{-1}	Ref.
			cb /r-EPS	HoPS/HePS	monosaccharide compositions	Mw (kDa)				
HT29	*L. acidophilus*	606	r-EPS	–	–	–	MTT	96	0.1–10	(Choi et al., 2006)
			cb-EPS	–	–	–	MTT/LDH	72	0.1–1	(Kim et al., 2010)
		BCRC 14079	r-EPS	–	–	–	MTT	72	0.7161	(Hsieh et al., 2016)
	L. brevis	TD4	cb-EPS	–	–	–	MTT	48	10.75 ± 1.034	(Mojibi et al., 2019)
	L. casei	SB27	r-EPS-LW1	HePS	galactose, glucose	25.10	MTT	48	0.01–0.05	(Di et al., 2017)
			r-EPS-LW2	HePS	galactose, glucose	12.34	MTT	48 72	0.1–0.2 0.05–0.1	
	L. helveticus	MB2-1	cb-EPS	HePS	glucose, mannose, galactose, rhamnose, arabinose	183	MTT	72	0.25	(Li, Xia et al., 2015)
	L. paracasei	TD3	cb-EPS	–	–	–	MTT	48	12.5 ± 1.12	(Mojibi et al., 2019)
	L. plantarum	70810	cb-EPS	HoPS	galactose	169.6	MTT	72	0.2	(Wang et al., 2014b)
		WLPL04	r-EPS	HePS	xylose, glucose, galactose	66.1	MTT	72	0.4–0.8	(Liu et al., 2017)
	L. rhamnosus	ATCC9595	r-EPS	HePS	galactose, glucose, rhamnose, mannose	43, 860	MTT	72	0.1–0.5	(Kim et al., 2006)
Caco2	*L. acidophilus*	10307	r-EPS	–	–	–	MTT/live and dead stain	48	4–5	(Deepak, Ramachandran et al., 2016)
		DSMZ 20079	r-EPS	HePS	glucose, fucose, glucuronic acid	–	neutral red dye/BrdU	48	1.237	(El-Deeb et al., 2018)
		BCRC 14079	r-EPS	–	–	–	MTT	72	0.7416	(Hsieh et al., 2016)
	L. helveticus	MB2-1	r-EPS-LHEPS1	HePS	galactose, glucose, mannose	208	MTT	72	0.4–0.6	(Li, Tang et al., 2015)
	L. plantarum	NRRL B-4496	r-EPS	HoPS	glucose	–	SRB	48	9.07	(Haroun et al., 2013)
		C70	r-EPS	HePS	arabinose, mannose, glucose, galactose	380	ab112118	72	< 5	(Ayyash, Abu-Jdayil, Itsaranuwat et al., 2020)
	L. reuteri	SHA101	r-EPS	–	–	–	CCK8	24	0.4–0.6	(Riaz Rajoka et al., 2019)
	L. rhamnosus	SHA111	r-EPS	–	–	–	CCK8	72	0.2–0.4	(Riaz Rajoka et al., 2018)
		SHA113	r-EPS	–	–	–	CCK8	72	0.4–0.6	(Riaz Rajoka et al., 2018)
	L. vaginalis	SHA110	r-EPS	–	–	–	CCK8	24	0.2–0.4	(Riaz Rajoka et al., 2019)
	P. pentosaceus	M41	r-EPS	HePS	arabinose, mannose, glucose, galactose	682.07	ab112118	72	< 5	(Ayyash, Abu-Jdayil, Olaimat et al., 2020)
	S. thermophilus	ASCC 1275	r-EPS	HePS	Mannose, galactose, glucose, glucosamine, galactosamine, glucuronic acid, ribose	–	MTT	72	0.4–0.8	(Li & Shah, 2016)
CX1	*L. acidophilus*	606	r-EPS	–	–	–	MTT	96	0.1–10	(Choi et al., 2006)
DLD1	*L. acidophilus*	606	r-EPS	–	–	–	MTT	96	0.1–10	(Choi et al., 2006)
HCT 15	*L. acidophilus*	10307	r-EPS	–	–	–	MTT/live and dead stain	48	4–5	(Deepak, Ramachandran et al., 2016)
HCT 116	*L. plantarum*	NRRL B-4496	r-EPS	HoPS	glucose	–	SRB	48	17.6	(Haroun et al., 2013)
CT26	*L. plantarum*	NCU116	r-EPS	HePS	galactosamine, glucosamine, glucose, mannose, glucuronic acid	384	CCK8	48	0.4–0.8	(Zhou et al., 2017)

Liver										
cell	Species	Strain	Form	Structure			method	T/h	IC_{50}/mg•mL^{-1}	Ref.
			cb /r-EPS	HoPS/HePS	monosaccharide compositions	Mw (kDa)				
HepG2	*L. helveticus*	MB2-1	cb-EPS	HePS	glucose, mannose, galactose, rhamnose, arabinose	183	MTT	72	1.52	(Li, Xia et al., 2015)
	L. lactis	NCR112	r-EPS	–	–	–	SRB	48	< 20	(Nguyen & Nguyen, 2014)
	L. plantarum	NRRL B-4496	r-EPS	HoPS	glucose	–	SRB	48	19.9	(Haroun et al., 2013)
		70810	cb-EPS	HoPS	galactose	169.6	MTT	72	0.4–0.6	(Wang et al., 2014b)
	S. thermophilus	ASCC 1275	r-EPS	HePS	Mannose, galactose, glucose, glucosamine, galactosamine, glucuronic acid, ribose	–	MTT	72	0.1–0.2	(Li & Shah, 2016)

(*continued on next page*)

Table 2 (*continued*)

Liver										
cell	Species	Strain	Form	Structure			method	T/h	IC_{50}/mg•mL^{-1}	Ref.
			cb /r-EPS	HoPS/HePS	monosaccharide compositions	Mw (kDa)				
		CH9	r-EPS-3a	HePS	fucose, ribose, rhamnose, arabinose, xylose, sorbose, glucose, galactose	1050	MTT	24	0.31375	(Sun et al., 2018)

Breast										
cell	Species	Strain	Form	Structure			method	T/h	IC_{50}/mg•mL^{-1}	Ref.
			cb /r-EPS	HoPS/HePS	monosaccharide compositions	Mw (kDa)				
	L. acidophilus	DSMZ 20079	r-EPS	HePS	glucose, fucose, glucuronic acid	–	neutral red dye/BrdU	48	1.756	(El-Deeb et al., 2018)
		NRRL B-4496	r-EPS	HoPS	glucose	–	SRB	48	24.2	(Haroun et al., 2013)
MCF7	*L. plantarum*	MTCC9510	r-EPS	HePS	mannose, glucose	–	MTT	24	1.0–10	(Ismail & Nampoothiri, 2013)
		C70	r-EPS	HePS	arabinose, mannose, glucose, galactose	380	ab112118	72	5–10	(Ayyash, Abu-Jdayil, Itsaranuwat et al., 2020)
	P. pentosaceus	M41	r-EPS	HePS	arabinose, mannose, glucose, galactose	682.07	ab112118	72	5–10	(Ayyash, Abu-Jdayil, Olaimat et al., 2020)

Other Organs										
cell	Species	Strain	Form	Structure			method	T/h	IC_{50}/mg•mL^{-1}	Ref.
			cb /r-EPS	HoPS/HePS	monosaccharide compositions	Mw (kDa)				
EAC mammary	*L. acidophilus*	P185	r-EPS	HePS	glucose, galactose, glucuronic acid	–	trypan blue	–	0.3–0.4	(Abd El Ghany et al., 2014)
	L. helveticus	MB2-1	r-EPS-LHEPS3	HePS	galactose, glucose, mannose	201	MTT	72	0.4–0.6	(Li, Tang et al., 2015)
BGC823 Gastric	*L. helveticus*	MB2-1	cb-EPS	HePS	glucose, mannose, galactose, rhamnose, arabinose	183	MTT	72	2.878	(Li, Xia et al., 2015)
	L. plantarum	70810	cb-EPS	HoPS	galactose	169.6	MTT	72	0.2–0.4	(Wang et al., 2014b)
Hela Cervix	*L. lactis*	NCR112	r-EPS	–	–	–	SRB	48	< 20	(Nguyen & Nguyen, 2014)
	L. plantarum	NRRL B-4496	r-EPS	HoPS	glucose	–	SRB	48	15.8	(Haroun et al., 2013)
PANC1 Pancreas	*L. rhamnosus*	ATCC9595	r-EPS	HePS	galactose, glucose, rhamnose, mannose	8600/43	MTT	72	0.5–1	(Kim et al., 2006)
MiaPaCa Pancreas	*L. plantarum*	RJF4	r-EPS	HePS	glucose, mannose	–	Alamar Blue Assay	–	0.1–1	(Dilna et al., 2015)
HEP2 Larynx	*L. plantarum*	NRRL B-4496	r-EPS	HoPS	glucose	–	SRB	48	34.7	(Haroun et al., 2013)

Abbreviation: BrdU, 5-bromo-2-deoxyuridine; CCK8, cell counting kit-8; cb-EPS, cell bound EPS; EAC, Ehrlich Ascites Carcinoma; HePS, heteropolysaccharides; HoPS, homopolysaccharides; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Mw, molecular weight; r-EPS, released EPS; SRB, sulforhodamine B.

600 μg/mL and 72 h incubation, the purified fraction LHEPS1 of *Lactobacillus helveticus (L. helveticus)* MB2-1 has a 56.34 % inhibition rate on human colon cancer Caco2 cells but only 4% inhibition rate on human colonic epithelial cells (Li, Tang et al., 2015).

2.4. Cell cycle arrest

Cell cycle is a series of events that happen in a cell to make it divide into two daughter cells. It has four sequential phases including G_1, S, G_2 and M. DNA replication occurs at S phase and mitosis occurs in M phase. G_1 and G_2 are the gap phases to separate S and M. G_0 represents a quiescent stage when cells at the G_1 phase reversibly withdraw from the

Table 3
Effects of LAB EPS on cell cycle distribution of cancer cell lines.

Lactic acid bacteria		Cancer	Cell Cycle Phase Proportion				Experimental conditions	Ref.
Species	Strain	Type	Apoptosis cell	G_0/G_1	S	G_2/M	Concentration/Time	
L. casei	X12/K11/M5/SB27	HT29		↑	↓	↓	500 μg/mL 48 h	(Di et al., 2018)
L. acidophilus	DSMZ 20079	Caco2	↑	↓	↓	↓	Not mentioned	(El-Deeb et al., 2018)
L. acidophilus	606	HT29		–	–		10 mg/mL	(Kim et al., 2010)
S. thermophiles	CH9	HepG2	↑	↑	↓	↓	313.75 μg/mL 24 h, 48 h	(Sun et al., 2018)

Note: ↑increase, ↓decrease, –no significant change, blank space on the form means data were not shown in the article.

cell cycle (Williams & Stoeber, 2012). Normally, cells with DNA damage actively halt progression to repair DNA breaks and adducts or undergo apoptosis, thus reducing the incidence rate of tumor development. However, mutations in cell cycle or apoptosis allow uncontrolled proliferation of these cells, leading to the malignant transformation (Kastan & Bartek, 2004). LAB EPS are documented to change the cell cycle distribution of cancer cell lines (summarized in Table 3). El-Deeb et al. (2018) observed a considerable arrest of cell cycle in the sub-G1 phase, which means more cell apoptosis occurs after LAB EPS treatment. Di et al. (2018) and Sun et al. (2018) found that the cytotoxic effects of LAB EPS against cancer cells was mediated *via* cell cycle arrest in G_0/G_1 phase, which refers to the prevention of G1 to S transition. However, Kim et al. (2010) found no influence of *L. acidophilus* 606's cb-EPS on the cell cycle of cancer cells since no significant alterations in G_0/G_1 and S phases were observed. More researches are needed to confirm this mechanism.

2.5. Anti-mutagen

High temperature cooked meat produces mutagenic compounds such as heterocyclic amines, and was investigated to be closely associated with coloractal cancer (Sinha, Kulldorff, Chow, Denobile, & Rothman, 2001). Various live LAB, especially *L. acidophilus*, show high anti-mutagenic activity by bonding or inhibiting the mutagens permanently (Caldini et al., 2005; Lankaputhra & Shah, 1998). 10 or 50 μg/mL purified component of bacteria EPS isolated from *L. casei* demonstrate inhibitory effects against 4-NQO (Liu et al., 2011). This anti-mutagenic effect is not attributed to the direct interaction between EPS and 4-NQO. In fact, the pre-treated EPS display a blocking effect by modulating the function of intestine cells, and the post-treated EPS can repair cell damage caused by 4-NQO (Liu et al., 2011).

2.6. Anti-oxidation

LAB EPS are proved to be anti-oxidative in tumor cell lines by a variety of assays including scavenging abilities on DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), hydroxyl and superoxide radicals, metal chelating activity as well as reducing power assay (2019, Abd El Ghany et al., 2014; Adebayo-Tayo & Fashogbon, 2020; Ayyash, Abu-Jdayil, Itsaranuwat et al., 2020; Choi et al., 2006; Deepak, Ramachandran et al., 2016; Dilna et al., 2015; Nguyen & Nguyen, 2014; Riaz Rajoka et al., 2018; Taylan, Yilmaz, & Dertli, 2019; Wang et al., 2014a; Wang, Zhao, Yang, Zhao, & Yang, 2015; Wang, Li et al., 2015; Xu et al., 2019). The higher the concentration, the better the anti-oxidative effect (Deepak, Ramachandran et al., 2016; Riaz Rajoka et al., 2019; Wang, Zhao et al., 2015). Reactive oxygen species (ROS) are generated in normal cells as an inevitable byproduct of oxidative processes. Cells produce antioxidants to resist the toxicity of ROS such as cell dysfunction, death, or malignant transformation (Manda, Nechifor, & Neagu, 2009). Due to the increased metabolic activity as well as many other tumourigenic events, tumor cells display a continuing increase in the intrinsic generation of ROS, which promotes tumor cell survival and proliferation, epithelial-to-mesenchymal transition and invasion, genomic instability and angiogenesis. A treatment using antioxidants to block ROS may be effective in delaying or even preventing the development of tumors (Liou & Storz, 2010). The production of ROS in HCT15 and Caco2 were significantly reduced after treated with 5 mg/mL EPS from *L. acidophilus* 10307 (Deepak, Ramachandran et al., 2016). Besides, administering 100 mg/kg EPS by gastric gavage attenuates the dimethylhydrazine caused colon oxidative injury by decreasing the oxidative stress product malondialdehyde along with increasing the anti-oxidant enzymes glutathione peroxidase and superoxide dismutase in cancer animal models (Zahran et al., 2017). The high anti-oxidant activity might be due to the chemical groups of EPS such as hydroxyl group (Dilna et al., 2015; Wang, Zhao et al., 2015), carbon-free radicals, (Wang, Zhao et al., 2015) and sulfated group (Wang et al., 2014a).

2.7. Anti-angiogenesis

5 mg/mL EPS from *L. acidophilus* 10307 significantly downregulated angiogenic genes but upregulated anti-angiogenic genes (Deepak, Ramachandran et al., 2016). The expression of vascular endothelial growth factor (*VEGF*) (Deepak, Ramachandran et al., 2016) was inhibited. VEGF is produced by many tumors to promote the formation of micro-vessels in and around the tumor. This inhibitory effect may be associated with the modulation of Hypoxia-inducible factor (HIF) signal pathway and tissue inhibitor metalloproteinases 3 (TIMP3) (Deepak, Ramachandran et al., 2016). VEGF is the master regulator of angiogenesis (the formation of new blood vessels), which mainly functions in vascular endothelial cells (Carmeliet, 2005; Ferrara & Adamis, 2016). The recruitment of blood vessels promotes the growth and progression of tumor by supplying oxygen and nutrients and removing the waste. Besides, it also sustains a favorable niche for cancer stem cells and acts as a channel for tumor cell metastasis and immune cell infiltration (Fukumura, Kloepper, Amoozgar, Duda, & Jain, 2018). After EPS treatment, the expression of *HIF1A* is significantly decreased under hypoxic conditions, whereas, *HIF2A* is increased (Deepak, Ramachandran et al., 2016). HIF1 is a transcription factor activated in a hypoxia-dependent manner. It is a heterodimer composed of one of three alpha subunits (HIF1A, HIF2A, HIF3A; also known as endothelial PER-ARNT-SIM domain protein 1) and an aryl hydrocarbon nuclear translocator (also known as HIF1B) (Huang & Bunn, 2003). The major HIF alpha isoforms required for induction of hypoxia-inducible genes (such as VEGF) are different in a cell type-dependent manner. HIF1A mainly acts on endothelial and breast cancer cells. Loss of HIF1A in endothelial cells significantly inhibits the growth of blood vessels in solid tumors with a decreased level of VEGF (Tang et al., 2004). HIF2A critically acts on renal carcinoma cells (Sowter, Raval, Moore, Ratcliffe, & Harris, 2003). Through angiopoietin 2-mediated pathway and delta-like ligand 4/Notch pathway, HIF2A null endothelial cells display an increased number of newly formed vessels but without effective remodeling, thus resulting in reduced overall vascular area, poor perfusion and low tissue oxygenation (Skuli et al., 2009, 2012). Additionally, EPS promote the expression of *TIMP3*. TIMP3 inhibits angiogenesis by blocking the binding of VEGF to VEGF receptor. Besides, by its named function to inhibit matrix metalloproteinases, TIMP3 can regulate matrix and thereby affect a variety of physiological processes such as proliferation and invasion of cancerous cells (Qi et al., 2003). However, another study of Deepak et al. found that the same EPS may promote angiogenesis by enhancing the expression of *EPO*, a factor that contributes to the erythropoiesis and increases the survival of the cancer cells (Deepak, Ram Kumar Pandian, Sivasubramaniam, Nellaiah, & Sundar, 2016). Very few articles reported the role of EPS in angiogenesis, and the studies were only at genetic level (Deepak, Ramachandran et al., 2016). The evidence till now is insufficient to prove that EPS do play an anti-cancer role by inhibiting angiogenesis.

2.8. Anti-inflammation

It has been indicated that inflammation and cancer have a strong relationship. Chronic inflammation and persistent infections at specific sites (particularly liver and bowel) greatly increase cancer risk (Taniguchi & Karin, 2018). Besides, tumor progression can also provoke inflammation due to tumor cell death by insufficient blood supply or microbial invasion by barrier deterioration (Taniguchi & Karin, 2018). Approximately 15–20 % of cancer deaths worldwide are due to underlying infections and inflammatory (Mantovani, Allavena, Sica, & Balkwill, 2008). Treatment with non-steroidal anti-inflammatory drugs reduces the incidence of specific cancers, for example, colon and breast cancer, and reduces cancer mortality (Mantovani et al., 2008).

The mucous variant strain of *L. rhamnosus* RW-9595M induced

higher levels of anti-inflammatory cytokine (interleukin 10 (IL10)) and lower levels of pro-inflammatory cytokines (TNFα and IL6) compared with its parental strain. EPS of the mucous variant are purified and added to the culture medium of macrophages in the presence of the parental strain. The immunosuppressive effects become even more remarkable, confirming the importance of EPS as an anti-inflammation bacterial component (Bleau et al., 2010). To elucidate the anti-inflammatory mechanisms of EPS, inflammatory cell model is constructed by lipopolysaccharide (LPS) acting immune cells (such as murine macrophage RAW264.7 cell line, human peripheral blood mononuclear cell (PBMC) and porcine small intestinal epithelial cell line (IPEC-J2)) (Chen, Wu, & Hu, 2019; Gao et al., 2017; Li & Shah, 2016; Vitlic et al., 2019; Wang et al., 2020). The nontoxic concentrations of EPS are adopted, under which EPS promote cell viability and proliferation of various immune cell types including RAW264.7 (Wang, Wu, Fang, Min, & Yang, 2018, 2020; Xu et al., 2019) and lymphocytes (Ismail & Nampoothiri, 2013). According to the sequence of LPS and EPS, the experiments can be divided into three kinds of experiments: (1) simultaneous LPS stimulation treatment; (2) pre-LPS stimulation treatment and (3) post-LPS stimulation treatment. Compared with the group that only treated with LPS, the pro-inflammatory cytokines including IL1β, IL6 and TNFα are significantly decreased and the anti-inflammatory IL10 is increased in all the three experiments (Chen et al., 2019; Gao et al., 2017; Li & Shah, 2016; Vitlic et al., 2019; Wang et al., 2020). The expression of pro-inflammatory *IL12* is also inhibited when incubating IPEC-J2 with 1 μg/mL *L. rhamnosus* GG EPS before LPS stimulation (Gao et al., 2017). Besides, EPS from *L. planetarium* JLAU103 reduces the production of nitric oxide (NO) and prostaglandin E2 by down-regulating the key enzymes in their synthesis including inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX2) (Wang et al., 2020). These molecular regulations may be mediated by the reduction of toll-like receptors (TLRs) including TLR2, TLR4, and TLR9 by EPS pretreatment (Gao et al., 2017; Vitlic et al., 2019). TLRs are single-pass membrane-spanning receptors on cell surface, which are targets for LPS (Kawai & Akira, 2007). All TLRs culminate in the activation of transcription factors such as nuclear factor kappa B (NF-κB) and activating protein-1 (AP-1), which translocate to the nucleus and trigger the gene expression of inflammation-related cytokines. NF-κB is a heterodimer composed of p65 and p50 subunits, which keeps inactive in the cytoplasm by interacting with its inhibitor IκBα. The phosphorylation of IκBα allowing NF-κB to be released into the nucleus. EPS suppress the activation of NF-κB by inhibiting IκBα phosphorylation (Gao et al., 2017; Wang et al., 2020). AP-1 is a basic region leucine zipper dimerization composed of c-Fos and c-Jun. AP-1 activation in TLR signaling is influenced by mitogen-activated protein kinase (MAPK) such as p38 (Kawai & Akira, 2007). It was found that the level of p-p38 is decreased when pretreating with 1 μg/mL *L. rhamnosus* GG EPS for 4 h (Gao et al., 2017). In summary, EPS block the TLR surface receptors on macrophages thus inhibits downstream MAPK and NF-κB signaling pathways and subsequently attenuates the production of inflammatory mediators (Fig. 3).

Immune cells play a vital role in cancer-related inflammation. The extrinsic stimuli (inflammation or infection) together with intrinsic stimuli (genetic alterations) act on tumor cells and activate a transcriptional program that resembles what occurs in inflammation (Mantovani et al., 2008). Transcription factors such as NF-κB, signal transducer and activator of transcription 3 (STAT3) and HIF1A in tumor cells are activated and lead to the production of pro-inflammatory cytokines, chemokines and growth factors (Mantovani et al., 2008). These molecules recruit immune cells like leukocytes and monocytes, and activate the transcription factors in immune cells, stromal cells, and tumor cells. In this way, more inflammatory mediators are produced and an inflammatory microenvironment is generated in tumors (Mantovani et al., 2008). Studies have proved that the same EPS can play two roles, suppressing inflammation of immune cells and inhibiting proliferation of tumor cells (Li & Shah, 2016; Xu et al., 2019). Fig. 3 demonstrated the crosstalk between cancer cells and immune cells, the sites that EPS inhibit during this process, as well as the changes of the inflammatory factors downstream. In tumor cell lines, EPS inactive NF-κB by inhibiting the gene expression of *NF-κB* and upregulating its inhibitor *IκBα* (El-Deeb et al., 2018; Zahran et al., 2017). Besides, they also reduce the

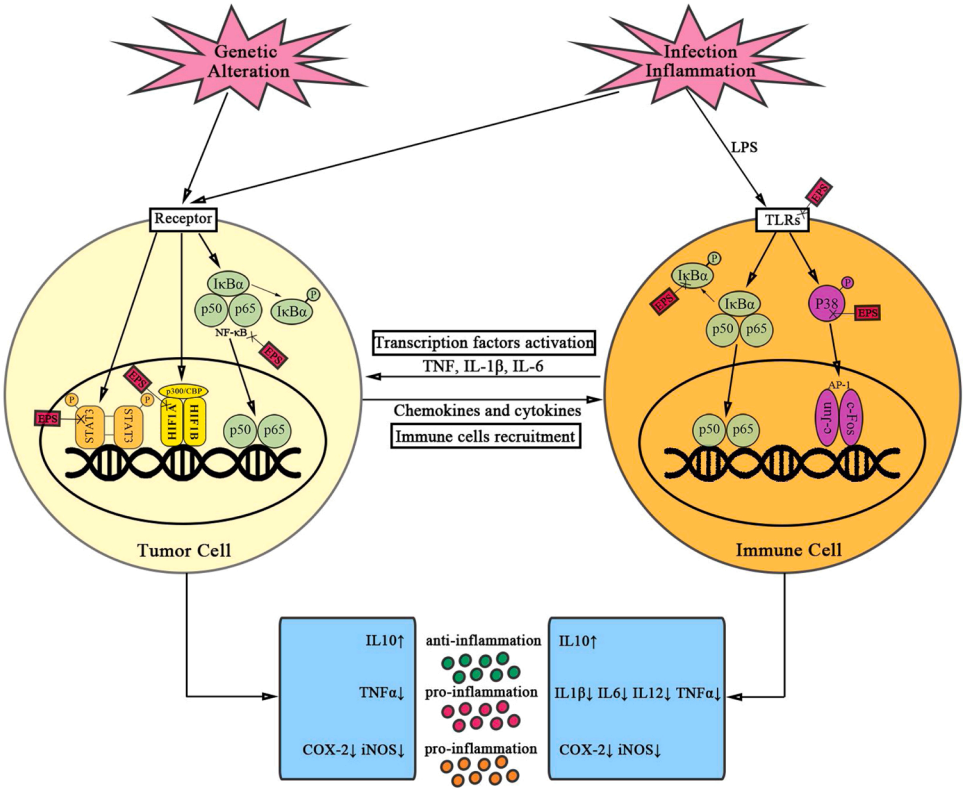

Fig. 3. Mechanisms by which LAB EPS regulate cancer-related inflammation. The crosstalk between cancer cells and immune cells contributes to the inflammatory microenvironment in tumors. EPS inhibit JAK/STAT3, NF-κb and HIF pathways in tumor cells as well as NF-κB and p38/MAPK in immune cells. The sites that EPS play an inhibitory role in these pathways are marked (EPS ⊣). After EPS treatment, the level of anti-inflammatory cytokine (IL10) is increased (↑). Meanwhile, the levels of pro-inflammatory cytokines such as TNFα in tumor cells and IL1β, IL6, IL12, TNFα in immune cells are significantly reduced (↓). The pro-inflammatory enzymes including COX-2 and iNOS are also decreased (↓), leading to a drop in the production of PGE2 and NO (↓).

phosphorylation of STAT3 (Zahran et al., 2017). STAT3 is a member of STAT family, which takes part in JAK/STAT pathway. It enters the nucleus and activates target genes after dimerization through phosphotyrosine-SH2 domain interaction (Bromberg et al., 1999). The downstream pro-inflammatory molecules of NF-κB and JAK/STAT pathways include TNFα, COX2, and iNOS, which are exhibited at a low level after EPS treatment (Sungur et al., 2017; Zahran et al., 2017). Tumor microenvironment also contains anti-inflammatory cytokines such as IL10 (Grivennikov & Karin, 2010), the production of which is enhanced after EPS treatment (Fig. 3) (Sungur et al., 2017). The decrease of *HIF1A* and the increase of *HIF2A* by EPS may reduce the production of NO. The loss of *HIF1A* reduces *INOS* expression, and thus decreases the production of NO, and the loss of *HIF2A* reduces arginase1 expression, and increases NO production (Branco-Price et al., 2012).

2.9. Signal pathway modulation

Cancer-related physiological functions mentioned above are regulated by signaling pathways at the molecular level. Dysfunction of signal transduction networks leads to tumorigenesis (Kolch, Halasz, Granovskaya, & Kholodenko, 2015). The effects of the variations in signaling pathways on tumor development depend on the functions of target genes. In addition to the signal paths already mentioned (NF-κB, JAK/STAT and HIF signal pathways), EPS also inhibits the production of β-catenin (belonging to canonical Wnt pathway) and the phosphorylation of p38 (belonging to MAPK pathway) (Zahran et al., 2017). Activation or genetic mutations of Wnt components activates the accumulation of β-catenin in the cytoplasm. Then β-catenin translocates into the nucleus and promotes the transcription of target genes including *JUN, C-MYC* and *cyclin D1*, which encode oncoproteins (Shang, Hua, & Hu, 2017). p38 leads to either cell survival or cell death when regulating tumor cell death (Koul, Pal, & Koul, 2013). When modulating tumor cell survival, activated p38 contributes to epithelial-to-mesenchymal transition, cell invasion and migration. When acting as an inhibitor, p38 takes part in oncogene-induced senescence, replicative senescence, DNA-damage responses and contact inhibition (Han & Sun, 2007). Just like the dual functions of p38, transforming growth factor β (TGFβ), the gene expression of which is enhanced by EPS, also acts as a double-edged sword in tumor (El-Deeb et al., 2018). TGFβ, with pleiotropic nature, functions as a tumor suppressor by inhibiting cell proliferation in early-stage tumors, but serves as an oncogenic factor by inducing progression and metastasis in advanced tumors (Seoane & Gomis, 2017). The dual function of these factors on cancer cells may depend on specific cell types, diverse stimuli and/or the activated isoform as well as the mediation functions of the components downstream. The gene expression of *p53* transcription factor is upregulated by EPS (El-Deeb et al., 2018). p53 signaling pathway is normally "off" but is activated in response to a variety of stress conditions such as DNA damage, oncogene activation, loss of normal cell contacts, and hypoxia (Bykov, Eriksson, Bianchi, & Wiman, 2018; Li et al., 2012; Muller & Vousden, 2013; Vogelstein, Lane, & Levine, 2000). The downstream target genes of p53 lead to cell cycle arrest, cell death by ferroptosis, senescence and apoptosis, the inhibition of blood vessel development as well as the maintenance of genetic stability. The loss of p53 occurs in nearly all human cancers, making it an important tumor suppressor (Bykov et al., 2018; Li et al., 2012; Vogelstein et al., 2000). None of the signal pathways functions independently. It is important to understand the crosstalk among these signaling pathways in a context-specific response comparing normal physiological and pathological conditions to better understand the role of this signaling pathway in tumor.

3. Possible factors influencing the anti-cancer effect of EPS

Among EPS-producing species, the most reported EPS with good anti-cancer effects are from *L. plantarum*, *L. acidophilus* and *L. helveticus*. Even from the same species, the inhibitory effects of EPS varied from strain to strain (Di et al., 2018; Sungur et al., 2017). Choi et al. (2006) tested many kinds of r-EPS from various lactobacilli and found that EPS from *L. acidophilus* 606 exhibited the most anti-cancer effects. There are limited studies investigating the relationship between LAB strains and their anti-cancer effects. Strain specific anti-cancer effects may be due to their genetic differences. Besides, the different adhesive ability of LAB strain to tumor cells may also take effects (Sungur et al., 2017).

EPS generally exist in two forms depending on their attachment degree to the cell surface. Cell bound EPS (cb-EPS) are tightly and covalently linked to the cell surface. While released EPS (r-EPS) are slime layers loosely attached to the cell surface or secreted into the extracellular matrix (Ruas-Madiedo & de los Reyes-Gavilán, 2005; Zeidan et al., 2017). Kim et al. (2006) found that r-EPS was more effective in anti-cancer activity against PANC-1 and HT29 than cb-EPS, both of which were isolated from *L. rhamnosus* ATCC 9595. The stronger effect of r-EPS may be due to its better water-solubility. The solubility in water influences the biological functions of natural polysaccharides, which is a vital advantage of r-EPS since most anticancer drugs are not water soluble (Ismail & Nampoothiri, 2013). However, from the studies of Wang et al. (2014a,b), r-EPS and cb-EPS obtained from *L. plantarum* 70810 seemed to have similar anti-proliferative effects towards BGC823 and HT29.

Studies on the factors that influence the anticancer effects of EPS need to be further carried out at the level of chemical structure. In supplemental Table 1, we summarized the structural characterizations such as monosaccharide composition, molecular weight (Mw) and chemical configuration of LAB EPS from the 41 included articles. According to the monosaccharide composition, EPS can be classified into two categories: homopolysaccharides (HoPS), containing only one kind of monosaccharide in the whole chain, and heteropolysaccharides (HePS), containing a variety of monosaccharides (Rahbar Saadat, Yari Khosroushahi, & Pourghassem Gargari, 2019). Most of the EPS mentioned in this review were HePS, and a few were HoPS. The type of the dominant monosaccharide greatly affects their biological functions. EPS from *L. delbrueckii* ssp. *Bulgaricus* B3, which have the highest mannose amount among the four kinds of studied EPS, displayed the highest apoptosis inducing effect on HT29 (Tukenmez et al., 2019). The presence of higher glucose might contribute to the anticancer effects of EPS (Li, Xia et al., 2015). *In vivo*, EPS from wild *L. delbrueckii* (glucose as the main monosaccharide) prolonged the lifespan of tumor induced mice more effectively than EPS from mutant *L. delbrueckii* (galactose as the main monosaccharide) (Adebayo-Tayo & Fashogbon, 2020). *In vitro*, *S. thermophilus* EPS-3a with a higher ratio of glucose exhibited the strongest inhibitory effects on HepG2 in comparison with EPS-1a and EPS-2a (Sun et al., 2018). Receptors on the surface of different cancer cells have different sensitivity to different monosaccharides (Tukenmez et al., 2019). Li et al. (2014,2015a) purified three EPS fractions from *L. helveticus* MB2-1. LHEPS-1, the most component of which was glucose, significantly inhibited the proliferation of Caco2. And LHEPS-2, the most component of which was mannose, demonstrated higher anti-proliferative activity against BGC823 but no inhibitory effect against Caco2.

The influence of Mw on the anti-cancer effect of EPS is controversial. Some studies discovered that EPS of high Mw tended to be more effective against cancer (Hassan, Ipsen, Janzen, & Qvist, 2003; Ooi & Liu, 2000; Peng, Zhang, Zeng, & Kennedy, 2005; Wasser, 2002). However, others believed that low Mw enabled EPS to pass through the cell membrane barrier to better exert biological functions (Li et al., 2016). After incubating with HT29 for 48 h, IC_{50} of r-EPS LW2 fraction (12.34 kDa) was estimated to be ranging from 0.1 to 0.2 mg/mL (Di et al., 2017). However, IC_{50} of *S. thermophilus* CH9 r-EPS-3a with a high Mw of 1050 kDa was at a similar concentration. It was measured to be 0.31375 mg/mL when functioning on HepG2 for 24 h (Sun et al., 2018).

The existence of specific structures such as uronic acid, sulfate groups (Li & Shah, 2016; Wang et al., 2014a), protein particles (Sun et al., 2018), triple-stranded helical conformation (Li, Xia et al., 2015),

1,3-linkages (Ismail & Nampoothiri, 2013), β-type glycosidic linkages (Wang et al., 2014a) and more side chains (Li, Tang et al., 2015) may contribute to the anti-cancer activity of EPS. It is a good idea to enhance the anti-cancer effects of EPS through chemical modification. The anti-tumor activities of EPS can be greatly promoted after being modified by acetylation, phosphorylation, carboxymethylation and sulfonation (Li & Shah, 2016; Wang, Zhao et al., 2015).

It is necessary to ensure the high purity of EPS and to report the purity accurately. The effects of the unwanted components tend to be unpredictable and unknown, leading to wrong evaluation of the anti-cancer effects of EPS. However, few studies emphasized the purity of EPS, which should be paid attention to in further experiments. The differences in the steps of isolation and purification methods affect the purity of the extracted EPS. We summarized the isolation and purification methods of LAB EPS with IC_{50} in Table 4. Among all the methods, anion-exchange chromatography and/or size-exclusion chromatography are recommended since they contribute to more highly pure samples (Ruas-Madiedo & de los Reyes-Gavilán, 2005). At least, there should be no absorption at 260 nm or 280 nm in the UV spectrum to confirm the absence of protein and nucleic acid (Ayyash, Abu-Jdayil, Itsaranuwat et al., 2020; Wang et al., 2014b, b, Zahran et al., 2017).

4. Conclusion and future prospective

In summary, the anti-cancer mechanisms of LAB EPS contain anti-proliferation, apoptosis induction, cell cycle arrest, mutagenicity inhibition, oxidative stress modulation, angiogenesis inhibition and inflammatory amelioration. Molecules that function on carcinogenesis such as p53, BCL2, β-catenin et al. are confirmed to be modulated by or interfered with EPS. However, the summarized mechanisms were all based on *in vitro* studies. It seems evident that the microbiological source and the targeted tumor cells do make a difference on the anticancer effects of EPS. However, based on current studies, no conclusion can be drawn on which bacterial source has the strongest effect and which kind of tumor cell is the most sensitive. These greatly increase the incomparability between different articles. Besides, the wide variety of extraction and isolation methods also contribute to the incomparability. Few studies mentioned the purity of EPS isolated from different methods, which is of great importance since high purity is beneficial to the analysis of structural information as well as the elimination of impurity interference. The influence of some structural characteristics of EPS such as monosaccharide composition and content, Mw, linkage type, branching degree et al. has begun to be known. Some specific structures that contribute to the anti-cancer activity have also been pointed out. Now, the biggest challenge is to correlate a specific EPS structure with specific anti-cancer mechanisms. The thorough understanding of the structure-function relationship would accelerate the development of EPS as a useful complementary medicine for the prevention and treatment of cancers, which can be expected in the near future.

Funding

None.

Table 4
Summarization of isolation and purification methods of LAB EPS with IC_{50}.

Removal of protein		Removal of undesirable compounds		EPS precipitation		EPS further purification		Ref.
EnclosedCircle1	TCA	EnclosedCircle1	Heat to inactive enzymes	EnclosedCircle1	Ethanol	EnclosedCircle1	Dialyse	
EnclosedCircle2	Protease	EnclosedCircle2	Evaporate to reduce volume	EnclosedCircle2	Acetone	EnclosedCircle2	Lyophilize	
EnclosedCircle3	phenol: chloroform: isoamyl (25:24:1)	EnclosedCircle3	Centrifuge to remove cells			EnclosedCircle3	Wash with ethanol	
		EnclosedCircle4	Filtration			EnclosedCircle4	anion-exchange chromatography and/or size-exclusion chromatography	
		EnclosedCircle5	Remove DNA with DNase I					
–		③④				②		(Choi et al., 2006)
②		③						(2010, Kim et al., 2006)
		③				②		(Haroun et al., 2013; Abd El Ghany et al., 2014)
–		③				③ ②		(Ismail & Nampoothiri, 2013)
		③④				②④		(Li, Tang et al., 2015)
–		③④				③		(Nguyen & Nguyen, 2014)
–		③				④ ②		(Wang et al., 2014a)
–		③				②		(Riaz Rajoka et al., 2018)
③		③		②		③ ②		(Dilna et al., 2015)
		③④				②④		(Li, Tang et al., 2015)
–		③②				②④		(Li, Xia et al., 2015)
		③				③		(Deepak, Ramachandran et al., 2016)
②		③				②		(Hsieh et al., 2016)
		③④				③ ②		(Li & Shah, 2016)
		③				②④		(Di et al., 2017)
②		③⑤				②		(Ayyash, Abu-Jdayil, Itsaranuwat et al., 2020; Liu et al., 2017)
		③				②		(Ayyash, Abu-Jdayil, Olaimat et al., 2020; Riaz Rajoka et al., 2019; Zhou et al., 2017)
		③				④		(El-Deeb et al., 2018)
②		③②				②		(Sun et al., 2018)
–		③				–		(Mojibi et al., 2019)

CRediT authorship contribution statement

Jiayi Wu: Conceptualization, Investigation, Writing - original draft. **Yuheng Zhang:** Conceptualization, Investigation, Writing - original draft. **Ling Ye:** Conceptualization, Supervision, Writing - review & editing. **Chenglin Wang:** Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.carbpol.2020.117308.

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