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Original Article

Molecular docking study of sea urchin (*Arbacia lixula*) peptides as multi-target inhibitor for non-small cell lung cancer (NSCLC) associated proteins

[Estudio de acoplamiento molecular de péptidos de erizo de mar (*Arbacia lixula*) como inhibidor de múltiples objetivos para proteínas asociadas al cáncer de pulmón de células no pequeñas (NSCLC)]

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Abstract

Context: Lung cancer is a type of cancer that causes the most deaths worldwide. The most common type of lung cancer is non-small cell lung cancer (NSCLC). Sea urchin (*Arbacia lixula*) has high potential as an anti-NSCLC agent.

Aims: To analyze the anticancer activity of peptides from *A. lixula* coelomic fluid in inhibiting the activity of NSCLC-related proteins.

Methods: Peptide modeling was performed using the PEP-FOLD3 web server. Proteins that have a crucial role in NSCLC progression were determined using KEGG pathway database. 3D protein structures such as EGFR, PI3K, BRAF V600E, and JAK3 were taken from the RCSB PDB database. Docking was performed using Autodock Vina software. Docking results analysis was carried out using Discovery Studio 2019 software.

Results: Some peptides bind to the active sites with low binding affinity. Peptide 10 binds to the active site of the EGFR with a binding affinity of -9 kcal/mol. Peptide 5 binds to the active sites of PI3K and BRAF V600E with binding affinity of -8.2 and -8.1 kcal/mol, respectively. Peptide 11 binds to the active site of JAK3 with a binding affinity of -8.1 kcal/mol. All of these peptides have lower binding affinity than ATP as the native ligand. Besides, these peptides also produce more hydrogen bonds than ATP, so they are predicted to be more stable.

Conclusions: Peptides 10, 5, and 11 have high potential as anti-NSCLC agents because they can inhibit the activity of proteins that play an essential role in the growth of NSCLC, namely EGFR, PI3K, BRAF V600E, and JAK3 through the competitive ATP inhibitor mechanism.

Keywords: *Arbacia lixula*; molecular docking; non-small cell lung cancer.

Resumen

Contexto: El cáncer de pulmón es un tipo de cáncer que causa la mayoría de las muertes en todo el mundo. El tipo más común de cáncer de pulmón es el cáncer de pulmón de células no pequeñas (NSCLC). El erizo de mar (*Arbacia lixula*) tiene un alto potencial como agente anti-NSCLC.

Objetivos: Analizar la actividad anticancerígena de péptidos del líquido celómico de *A. lixula* en la inhibición de la actividad de proteínas relacionadas con NSCLC.

Métodos: El modelado de péptidos se realizó utilizando el servidor web PEP-FOLD3. Las proteínas que tienen un papel crucial en la progresión del NSCLC se determinaron utilizando la base de datos de la vía KEGG. Las estructuras de proteínas 3D como EGFR, PI3K, BRAF V600E y JAK3 se tomaron de la base de datos RCSB PDB. El acoplamiento se realizó utilizando el software Autodock Vina. El análisis de los resultados del acoplamiento se llevó a cabo utilizando el software Discovery Studio 2019.

Resultados: Algunos péptidos se unen a los sitios activos con baja afinidad de unión. El péptido 10 se une al sitio activo del EGFR con una afinidad de unión de -9 kcal/mol. El péptido 5 se une a los sitios activos de PI3K y BRAF V600E con una afinidad de unión de -8,2 y -8,1 kcal/mol, respectivamente. El péptido 11 se une al sitio activo de JAK3 con una afinidad de unión de -8,1 kcal/mol. Todos estos péptidos tienen menor afinidad de unión que el ATP como ligando nativo. Además, estos péptidos también producen más enlaces de hidrógeno que el ATP, por lo que se prevé que sean más estables.

Conclusiones: Los péptidos 10, 5 y 11 tienen un alto potencial como agentes anti-NSCLC porque pueden inhibir la actividad de proteínas que juegan un papel esencial en el crecimiento del NSCLC, a saber, EGFR, PI3K, BRAF V600E y JAK3 a través del mecanismo inhibidor de ATP competitivo.

Palabras Clave: acoplamiento molecular; *Arbacia lixula*; cáncer de pulmón de células no pequeñas.

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INTRODUCTION

Lung cancer is the type of cancer that causes the most deaths worldwide (18.4% of all cancer deaths) in 2018. This type of cancer causes more deaths than breast, colorectal, and cervical cancer (Bray et al., 2018). In 2012, 1.6 million people died from lung cancer worldwide and were expected to continue growing (Didkowska et al., 2016). In 2018, most cancer cases were lung cancer, which reached 14% of all cancer cases globally, and the mortality rate was 22% (Ferlay et al., 2019). Lung cancer is divided into two subtypes: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The percentage of NSCLC reaches 85% (Jordan et al., 2017). NSCLC occurs due to the presence of mutated and overexpressed proteins that cause uncontrolled cell division.

Epidermal growth factor receptor (EGFR), phosphoinositide 3-kinase (PI3K), Janus kinase 3 (JAK3) and B-type Raf kinase (BRAF) are proteins that play a role in the growth and development of lung cancer, NSCLC subtype (Lee et al., 2011; El-Telbany and Ma, 2012; Hu et al., 2014). EGFR is a protein family of tyrosine kinase receptor that is overexpressed in 40-80% of cases of NSCLC (Suzuki et al., 2005). After binding to the EGF ligand, EGFR will activate the PI3K/Akt/mTOR pathway, the BRAF pathway, and the JAK-STAT pathway that plays an essential role in cell proliferation and growth (Andl et al., 2004; Liu et al., 2013; Singh et al., 2019). Therefore, compounds that can inhibit EGFR activity from fighting cancer were developed, such as gefitinib and lapatinib (Brehmer et al., 2005; Wainberg et al., 2010). PI3K is the central protein in the PI3k/Akt1/mTOR pathway. This pathway is overactivity in lung cancer characterized by overexpression of phosphorylated Akt1 in 50-70% of cases of NSCLC (Yip, 2015). The PI3K-Akt pathway plays an important role in cell survival by inactivating downstream apoptogenic factors (Zhuang et al., 2011). BRAF is a central protein in the BRAF pathway that regulates cell growth. Around 50% of NSCLC lung cancer patients have a V600E mutation in the BRAF protein (Bustamante and Otterson, 2019). BRAF V600E can upregulate anti-apoptotic proteins such as MCL-1, which prevents cell apoptosis (Kawakami et al., 2016). Therefore, one of the best strategies to inhibit lung cancer cell growth is to inhibit the activity of BRAF V600E mutation (O'Leary et al., 2019). JAK3 is the central protein in the JAK-STAT pathway, where it is overactivated by up to 65% in NSCLC cases. The overactivity of this pathway causes cells to experience uncontrolled growth (Hu et al., 2014; Groner and von Manstein, 2017). The JAK-STAT pathway also mediates antiapoptotic signaling by regulating the activity of the bcl-xL, bcl-2, and Bad proteins (Negoro, 2000). Therefore, inhibition of these four proteins will inhibit the three pathways that play a role in cancer cell growth, namely the PI3K/Akt1/mTOR pathway, the BRAF/MAPK pathway, and the JAK-STAT pathway. This research will explore peptides from sea urchins to stop the activity of these four proteins.

Sea urchin (*Arbacia lixula*) has high potential as an anti-lung cancer agent. Many sea urchins are reported to have anticancer activity. Previous research stated that *A. lixula*'s coelomic fluid extract could inhibit the cycle cell of triple-negative breast cancer (Luparello et al., 2020). *A. lixula* has a high astaxanthin content where this compound has activity in inhibiting the proliferation and migration of breast cancer cells (Cirino et al., 2017; McCall et al., 2018). *Diadema savigni* CH_2Cl_2 extract could induce apoptosis in several cell lines such as HL-60 (leukemia cell line), PC-3 (prostate cancer cell line), and SNU-C5 (colorectal cancer cell line) (Thao et al., 2015). In the current study, we used the *silico* molecular docking method to analyze the interactions between NSCLC-related proteins with peptides from *A. lixula* coelomic fluid so that the potential of these peptides in inhibiting the activity of these proteins will be identified.

The most common therapeutic methods to treat lung cancer are chemotherapy and radiotherapy, but both have many adverse side effects for patients, such as shortness of breath, bleeding, fever, hair loss, and radiation pneumonitis (Islam et al., 2019; Mohan et al., 2019). Therapeutic agents hav-

ing single-target properties sometimes show a lack of efficacy and occur drug-resistant in the cancer cell because of mutations in the target protein (Lim et al., 2017). Hence, a multi-target agent is needed to overcome these problems (Ramsay et al., 2018). There is no research discussed the peptides of *A. lixula* as anti-lung cancer, even though the peptide has several advantages over compounds such as being more selective, low toxicity, and not causing accumulation in the tissue because it is easily metabolized (Craik et al., 2013). This study provides the potential of peptides in the coelomic fluid of *A. lixula* as a multi-target inhibitor of proteins that have significant roles in three important pathways in NSCLC progression. Besides, an alternative lung cancer drug with minimal side effects is needed that targets more than one protein. This study aims to analyze the potential of the peptides from the coelomic fluid extract of *A. lixula* in inhibiting lung cancer growth by inhibiting the activity of EGFR, PI3K, BRAF V600E, and JAK3 proteins.

MATERIAL AND METHODS

Ligands preparation

The peptides used in this study are based on Sciani et al. (2016) research, namely peptides measuring 5-7 amino acids (Table 1). The 3D structure of the peptides was modelled using the PEP-FOLD3 web server (https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) with the de novo structure prediction method (Lamiable et al., 2016). The 3D adenosine triphosphate (ATP) structure as a native ligand was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) with CID: 5957. These peptides and ATP were prepared by minimizing conformation energy using PyRx 8.0 software (Dallakyan and Olson, 2015).

Protein's preparation

Lung cancer-related proteins were determined using the KEGG pathway database (https://www.genome.jp/kegg/pathway.html) then selected the central protein in PI3K/Akt1/mTOR pathway, JAK/STAT pathway, and BRAF/MAPK pathway, which have a role in the growth of NSCLC lung cancer. The 3D structure of EGFR kinase domain (1XKK), BRAF V600E (4R5Y), JAK3, and PI3K (1E90) were obtained from PDBJ database (https://pdbj.org/). The proteins were prepared using the Biovia Discovery Studio 2019 software (Dassault Systèmes Biovia, San Diego, California, USA) by removing the contaminant molecules.

Table 1. Peptides from sea urchin (*A. lixula*) coelom fluid.

Peptide code	Peptide sequence	Peptide code	Peptide sequence
Peptide 1	LSDCL	Peptide 11	MTVNGASVTN
Peptide 2	LAPAA	Peptide 12	GDKGSTAGSNH
Peptide 3	LVTELL	Peptide 13	MTKYAATGVTN
Peptide 4	DGHCGAD	Peptide 14	TSTLLFDAHVT
Peptide 5	HSGECSF	Peptide 15	EDQLVVKEVETF
Peptide 6	VTLMMSS	Peptide 16	APAEYLPVELPLYWY
Peptide 7	FVKVEVLPQ	Peptide 17	EWMEGGDVDVENARA
Peptide 8	REGSVCVEH	Peptide 18	KTGGGGVSGGSAGDH
Peptide 9	VAKGSPDLNK	Peptide 19	YMAAGASSSSSTKVVQK
Peptide 10	FVEQVLVEPQ		

Table 2. ATP binding pocket position and grid setting for specific docking.

Proteins	ATP binding pocket	Reference	Grid	
			Center	Dimension
EGFR kinase domain	Leu718, Val726, Gly745, Leu788, Gly796, Cys797, Leu844, Asp855	(Liao et al., 2011)	X: 16.3451 Y: 32.3674 Z: 35.9235	X: 31.3579 Y: 30.0286 Z: 34.7935
PI3K	Ala805, Ser806, Trp812, Lys890, Asp950, Asp964	(Walker et al., 2000)	X: 19.2311 Y: 68.9103 Z: 18.4851	X:46.6013 Y:39.4410 Z:44.5788
BRAF V600E	Ile463, Val471, Ala481, Lys483, Ile527, Leu514, Thr529, Gln530, Trp531, Cys532, Gly534, Asn581, Phe583	(Luoet et al., 2008)	X: 13.5842 Y: 16.5716 Z: -5.4844	X: 28.3015 Y: 40.8908 Z: 37.7227
JAK3	Leu828, Val836, Ala853, Lys855, Met902, E903, Leu905, Leu956, Ala966	(Thomas et al., 2015)	X: 12.7557 Y: -8.5375 Z: 11.3042	X: 29.6989 Y: 33.4322 Z: 28.380

Molecular docking and visualization

Specific protein-ligand docking was performed using Autodock Vina (Trott and Olson, 2010) integrated in PyRx 8.0 (Dallakyan and Olson, 2015) to find peptides that could potentially inhibit the activity of proteins, which have lower binding affinity values than ATP. Autodock Vina is a software for flexible docking, allowing flexibility of the ligands for more accurate docking results (Trott and Olson, 2010). Specific docking method is done by setting the grid box to cover only the ATP binding pocket of the proteins (Table 2). Analysis of binding site and chemical bonds formed between proteins and ligands were carried out using the Biovia Discovery Studio 2019 software (Dassault Systèmes Biovia, San Diego, California, USA).

RESULTS

NSCLC related proteins-peptide docking

The protein-peptide docking aims to predict the ability of the peptides from sea urchin to inhibit the activity of proteins related to NSCLC viewed from the binding affinity and binding position point of view. Binding affinity is defined as the strength of the interaction, which can be used to predict whether an interaction between two molecules can form or not. The lower binding affinity value, the stronger and more stable the interaction between molecules (Kastritis and Bonvin, 2013; Pantsar and Poso, 2018). The peptides that bind to the protein's active site with the lowest binding affinity value and below the ATP binding affinity are predicted to inhibit the protein activities. Docking results show that all peptides bind to the ATP binding pocket, which is the active site of the EGFR, PI3K, BRAF V600E, and JAK3 proteins (Fig. 1). Peptide 10 binds to the ATP binding pocket of EGFR with the lowest binding energy, which is -9 kcal/mol lower than ATP. Peptide 5 binds to PI3K and BRAF V600E active site with the lowest binding affinity of -8.2 kcal/mol and -8.1 kcal/mol, respectively. Peptide 11 binds to the active site of JAK3 with the binding affinity of -8.1 kcal/mol (Table 3). Therefore, by binding to the proteins' active site and having lower binding energy than ATP, those peptides are predicted to be able to inhibit protein activities by ATP competitive inhibitory mechanism.

EGFR-peptides docking

The docking results between the kinase domain of EGFR and the peptides showed that peptide 10 (FVEQVLVEPQ) has a lower binding affinity than ATP. Peptide 10 also binds to the same four residual amino acids as ATP, which are Gly721, Lys745, Arg841, and Asn842.

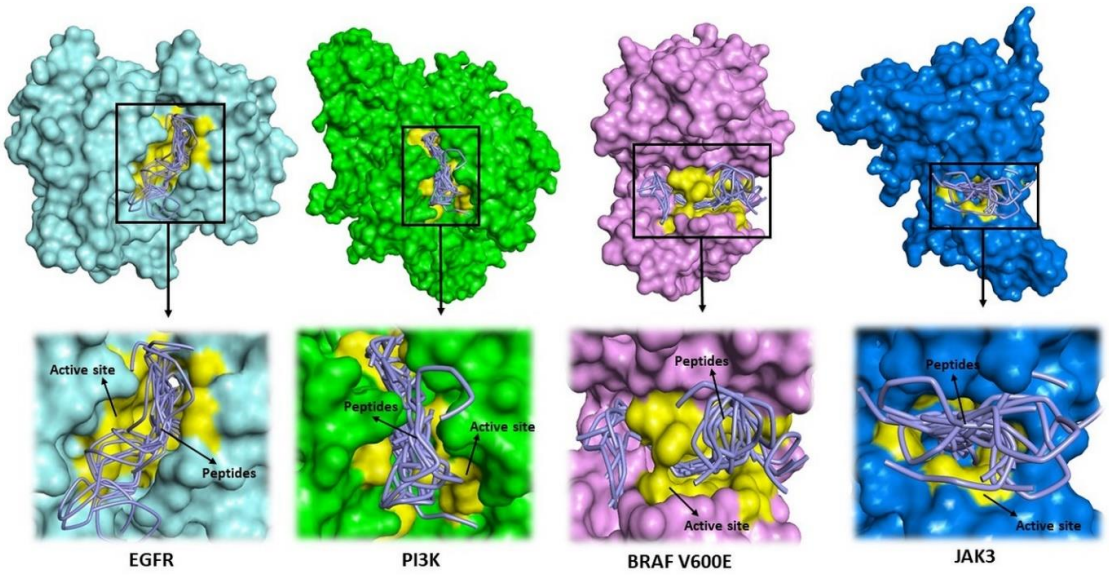

Figure 1. All peptides bind to the active site of the proteins.

Table 3. Several peptides such as peptides 10, 5, and 11 have lower binding affinity values than ATP as a native ligand when interacting with proteins.

Ligand	Peptide sequence	Binding affinity (kcal/mol)			
		EGFR	PI3K	BRAF	JAK3
ATP (Native ligand)	-	-8.9	-7.7	-6.9	-7.7
Peptide 1	LSDCL	-7.4	-7.3	-6.6	-7.3
Peptide 2	LAPAA	-8.5	-7.6	-7.4	-8
Peptide 3	LVTELL	-6.9	-.7.7	-6.6	-7.1
Peptide 4	DGHCGAD	-7.7	-7.5	-7.2	-7.7
Peptide 5	HSGECSF	-7	-8.2	-8.1	-7.5
Peptide 6	VTLMMSS	-7.4	-6.4	-6.9	-6.7
Peptide 7	FVKVEVLPQ	-2.2	-8	-7.3	-7.8
Peptide 8	REGSVCVEH	-7.6	-7.2	-6	-6.2
Peptide 9	VAKGSPDLNK	-7.2	-7	-6.2	-7.3
Peptide 10	FVEQVLVEPQ	-9	-7.8	-8.1	-6.8
Peptide 11	MTVNGASVTN	-6.9	-7.6	-6.3	-8.1
Peptide 12	GDKGSTAGSNH	-6.4	-7.2	-8	-5.9
Peptide 13	MTKYAATGVTN	-7.1	-7.5	-6.3	-7.2
Peptide 14	TSTLLFDAHVT	-5.3	-6.9	-6.2	-6.8
Peptide 15	EDQLVVKEVETF	-7.2	-6.7	-6.9	-6.8
Peptide 16	APAEYLPVELPLYWY	-8.5	-7.7	-7.1	-7.4
Peptide 17	EWMEGGDVDVENARA	-6.9	-6.8	-6.7	-6.4
Peptide 18	KTGGGGVSGGSAGDH	-6.5	-6.8	-7.2	-5.7
Peptide 19	YMAAGASSSSSTKVVQK	-7.5	-7.6	-5.9	-7.5

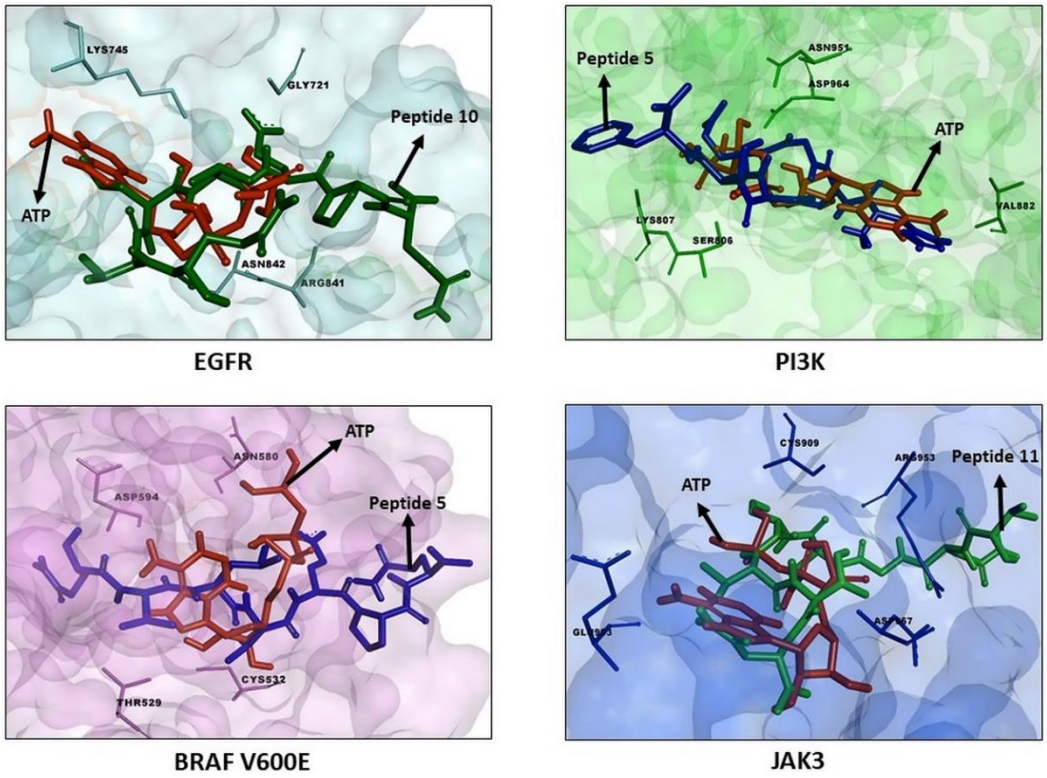

Figure 2. Peptides 10, 5, and 11 bind to the ATP binding pocket so that they are ATP competitive inhibitors.

On the other hand, peptide ten also form more chemical interactions than ATP that consists of 6 hydrogen bonds (Lys745, Phe723, Cys797, Tyr801, Tyr998, Glu906) and nine hydrophobic interactions (Gly721, Ala743, Arg841, Leu718, Leu792, Met793, Asn842, Phe997, Lys728). Hence, interactions at the same location as ATP with lower binding affinity means that peptide 10 has a high potential to be used as an ATP competitive inhibitor for EGFR (Fig. 2 and Table 4). Moreover, peptide ten also forms hydrogen bonds with Lys745 residue, a crucial amino acid residue for the binding of EGFR with ATP (Pao and Miller, 2005).

PI3K-peptides docking

The docking results between PI3K and peptides showed that peptide 5 (HSGECSF) binds to the ATP binding pocket with the lowest binding affinity (-8.2 kcal/mol). This peptide also formed bonds in the five residues that are the same as ATP, involve Ser806, Lys807, Val882, Asn951, and Asp964. Interaction between peptide 5 and PI3K is also predicted to be more stable because it produces more chemical bonds that are 13 interactions consist of 10 hydrogen bonds (Lys808, Val882, Ala885, Asp964, Asp950, Ser806, Lys807, Gln893, Asn951, Asp964) and three hydrophobic bonds (Trp812, Ile881, Ala885). These results showed that peptide 5 is predicted to inhibit PI3K activity by blocking ATP binding to the protein (Fig. 2 and Table 4).

BRAF-peptides docking

Peptide 5 also interacted with BRAF V600E protein with good affinity. The docking results showed that peptide 5 binds to the ATP binding pocket with the lowest binding affinity value and much lower than ATP, that is -8.1 kcal/mol. Peptide 5 binds in the same residue as ATP that in Thr529, Cys532, Asn580, and Asp594 residue. Interaction between BRAF V600E with peptide 5 is also more stable than ATP because it produces more chemical interactions consist of seven hydrogen bonds (Cys532, Thr529, His574, Asp594, Ala497, Asn500, Glu501) and four hydrophobic

interaction (Trp531, Asn580, Cys532, Val504). This result showed that peptide 5 has a high potential to inhibit BRAF V600E activity as a competitive inhibitor (Fig. 2 and Table 4).

JAK3-peptides docking

The docking results showed peptide 11 (MTVNGASVTN) binds to the ATP binding pocket with the lowest binding affinity value and lower than ATP, which is -8.1 kcal/mol. Peptide 11 forms bonds with the same four amino acids as ATP, namely Glu903, Cys909, Arg953, and Asp967. Peptide 11 forms nine hydrogen bonds (Glu903, Cys909, Arg911, Asp912, Asp949, Arg953, Asp967, Ser989) and seven hydrophobic interactions (Ala853, Leu828, Leu956, Ala951, Trp993, Pro990, Phe992). Based on the docking results, peptide 11 binds to the place where ATP binds to JAK3 so that it has a high potential to act as a JAK3 inhibitor. The interaction between JAK3 and blocked ATP will result in JAK3 unable to phosphorylate STAT3 and the JAK-STAT signaling pathway will stop (Fig. 2 and Table 4).

DISCUSSION

Epidermal growth factor receptor (EGFR) is a receptor responsible for activating several pathways in NSCLC (Tokumo et al., 2005). EGFR will be activated when it binds with EGF in the Ligand Binding Domain (LBD) that causes dimerization between EGFR proteins (Lemmon and Schlessinger, 2010). The kinase domain will then undergo autophosphorylation and activate several pathways that have a role in cell growth (Ciardiello et al., 2004). EGFR overexpression cause lung

Table 4. Protein-peptide interactions in detail.

Protein	Ligand	Binding affinity (kcal/mol)	Chemical interaction	Amino acid
EGFR	ATP	-8.9	Hydrogen bond	Gly721, Lys745, Arg841, Asn842, Asp855
			Hydrophobic interaction	-
	Peptide 10	-9	Hydrogen bond	Lys745, Phe723, Cys797, Tyr801, Tyr998, Glu906
			Hydrophobic interaction	Gly721, Ala743, Arg841, Leu718, Leu792, Met793, Asn842, Phe997, Lys728
PI3K	ATP	-7.7	Hydrogen bond	Ser806, Lys807, Val882, Lys833, Asn951, Asp964
			Hydrophobic interaction	-
	Peptide 5	-8.2	Hydrogen bond	Lys808, Val882, Ala885, Asp964, Asp950, Ser806, Lys807, Gln893, Asn951, Asp964
			Hydrophobic interaction	Trp812, Ile881, Ala885
BRAF	ATP	-6.9	Hydrogen bond	Thr529, Cys532, Phe595, Ser536, Asn580, Asp594
			Hydrophobic interaction	-
	Peptide 5	-8.1	Hydrogen bond	Cys532, Thr529, His574, Asp594, Ala497, Asn500, Glu501
			Hydrophobic interaction	Trp531, Asn580, Cys532, Val504
JAK3	ATP	-7.7	Hydrogen bond	Phe833, Glu903, Cys909, Arg953, Asp967
			Hydrophobic interaction	-
	Peptide 11	-8.1	Hydrogen bond	Glu903, Cys909, Arg911, Asp912, Asp949, Arg953, Asp967, Ser989
			Hydrophobic interaction	Ala853, Leu828, Leu956, Ala951, Trp993, Pro990, Phe992

cancer and EGFR overexpression occur in 89% of NSCLC patients (Prabhakar, 2015). One of the most effective strategies to stop EGFR activity is to block its interactions with ATP, so EGFR cannot activate several pathways after activating (Yun et al., 2008). Inhibitory agents targeted at EGFR have to bind with EGFR ATP binding pocket that is located in Leu718, Val726, Gly745, Leu 788, Gly796, Cys797, Leu844, and Asp855 residual amino acids so that they can compete with ATP (Liao et al., 2011). Previous research reports that the inhibition of EGFR activity can inhibit cancer cells' growth with several mechanisms. EGFR inhibited by gefitinib downregulates survivin's expression, an anti-apoptotic protein that means it can induce apoptosis on several NSCLC cell lines and can induce apoptosis in breast cancer cells (Campiglio et al., 2004; Okamoto et al., 2010). EGFR inhibited by PD153035 can increase the expression of several genes that stimulate inflammation, apoptosis, and cervical cancer cell invasion (Woodworth, 2005). FR18 that inactivates EGFR by blocking the binding of EGFR with EGF can induce apoptosis in HT29 colorectal cancer cell lines (Calonghi et al., 2007). However, EGFR inhibitors are sometimes not sufficient because there is a resistance mechanism. Therefore, other pathways are also needed to be inhibited (Glaysher et al., 2013).

Phosphoinositide 3-kinase (PI3K) is one of the central proteins in the PI3K/Akt pathway, which is activated by EGFR (Yip, 2015). After being activated, PI3K will phosphorylate PIP2 into PIP3, followed by activation of Akt1 and other downstream proteins (Hemmings and Restuccia, 2012). PI3K has overexpression in lung cancer cells that cause uncontrolled cell survival (Yamamoto et al., 2008). Therefore, one of the strategies to stop the survival of lung cancer cells is to inhibit this protein's activity. This protein's activity can be inhibited by blocking ATP binding to PI3K, so there is no conversion of PIP2 to PIP3. Hence, a molecule that can bind to PI3K in the ATP binding pocket is needed to prevent PI3K bindings with ATP. The ATP binding pocket of the PI3K protein is located around the residue of Ala805, Ser806, Trp812, Lys890, Asp950, and Asp964 (Walker et al., 2000). PI3K inhibition in previous studies was reported that can be inhibited the survival of various types of cancer cells. Inhibition PI3k by pilarasilib can reduce phosphorylation of Akt, PRAS40, 4EBPI, and S6 that involve in tumor tissue and inhibit MEK/ERK pathway in NSCLC patients (Chen et al., 2020). ATP competitive inhibitor, flupentixol dihydrochloride can induce apoptotic of lung cancer cell line A549 and H661 (Dong et al., 2019). However, PI3K inhibition is not predicted to be sufficient to inhibit the survival of NSCLC cancer cells because previous studies report that inhibition of PI3K/Akt pathway actually induces other pathways such as MET and STAT3 pathway (Bian et al., 2018).

B-type Raf kinase (BRAF) is a protein serine/threonine kinase that has a crucial role in the BRAF pathway that regulates cell growth, proliferation, survival, and differentiation (Leonetti et al., 2018). Mutation in the BRAF protein causes the pathway overactivity and permanent activation. BRAF mutation in exon 5 is a substitution of amino acid valine to glutamate at amino acid number 600 (V600E). This mutation leads to increased kinase domain activity of BRAF protein (Tan et al., 2008). This mutation is commonly found in NSCLC cases (O'Leary et al., 2019). Therefore, BRAF V600E inhibitor essential to inhibit the growth of NSCLC. One of the strategies is to inhibit phosphorylation activity by blocking the bond between BRAF and ATP. Inhibitor molecule must bind to BRAF V600E in the ATP binding pocket located around the residue of Ile463, Val471, Ala481, Lys483, Ile527, Leu514, Thr529, Gln530, Trp531, Cys532, Gly534, Asn581, and Phe583 (Luo et al., 2008). Previous studies reported that inhibition of BRAF V600E activity able to prevent cancer cell growth. BRAF V600E inhibited with vemurafenib resulted in apoptotic of various lung cancer cell lines, cell cycle arrest in the G1 phase, and increased BIM (pro-apoptotic) expression (Joshi et al., 2015). However, 30% of the total patients tested were resistant (Planchard et al., 2013). This is thought to be due to escaping via another pathway. In a previous study, colon cancer cells inhibited with vemurafenib induces activation of Akt (Prahallad et al., 2012). It is estimated that inhibit-

ed BRAF V600E at NSCLC cells escape via EGFR/PI3K/Akt pathway.

Janus kinase 3 (JAK3) and signal transducer and activator of transcription 3/5 (STAT3/5) is the main protein in the JAK-STAT signaling pathway. This pathway has an essential role in the regulation of cell survival and angiogenesis (Groner and von Manstein, 2017). The overactivity of the JAK-STAT pathway promotes the transformation of tumor cells into malignant (Thomas et al., 2015). Overactivity of this pathway occurs in 22-65% of NSCLC cases. Therefore, the JAK-STAT signaling pathway is a critical mediator for NSCLC (Hu et al., 2014). STAT3/5 will be active as a transcription factor after being phosphorylated by JAK3. Therefore, one strategy to inhibit the JAK-STAT signaling pathway is to stop the activity of JAK3 and STAT3/5 cannot become a transcription factor (Li et al., 2015). When phosphorylating STAT3/5, JAK3 needs to bind with ATP to transfer the phosphate to STAT3/5. Therefore, one way to inhibit JAK3 is blocking its interaction with ATP with the compound that binds to the ATP binding pocket of JAK3, which is located between the residues of Leu828, Val836, Ala853, Lys855, Met902, E903, Leu905, Leu956, and Ala966 (Thoma et al., 2011). Previous studies have stated that JAK-STAT pathway inhibition can inhibit the growth of various cancer cells. JAK-STAT pathway inhibited by AZD1480 can reduce the proliferation of head and neck squamous cell carcinoma (HNSCC) *in vitro* and *in vivo* (Sen et al., 2015). JAK3 inhibition by AG490 can induce apoptosis and cell cycle arrest in anaplastic large cell lymphoma (ALCL) (Amin et al., 2003). JAK3/STAT3 pathway inhibition can also induce apoptosis and cell cycle arrest in colon carcinoma cells (Lin et al., 2005).

We designed a pathway to illustrate the role of peptides in inhibiting three pathway activity in the NSCLC progression. Peptides from *A. lixula* based on docking results can inhibit three NSCLC-related pathways. Based on docking results, peptide 10 could inhibit EGFR activity. EGFR is an essential protein in the growth of lung cancer cells. EGFR activation will activate several signaling pathways such as PI3K/Akt pathway, BRAF pathway, and JAK-STAT pathway (Liu et al., 2013; Sun et al., 2016; Singh et al., 2019). The inhibition of EGFR activity will stop the activation of the three pathways. However, treatments targeting EGFR are sometimes unsuccessful due to resistance resulting from mutations in 10-30% of NSCLC patients (Stewart et al., 2015). That issue could be overcome by inhibiting the activity of other proteins that have a role in the growth of NSCLC. In this study, the peptides can inhibit proteins that play an essential role in the pathways activated by EGFR. Peptide 5 inhibits PI3K and BRAF V600E protein activity. PI3K is activated by EGFR converts PIP2 to PIP3. Then PI3K recruits the PDK1 protein to phosphorylate Akt. Akt will phosphorylate its target proteins and resulting in tumor cell growth and survival (Hemmings and Restuccia, 2012). Inhibition of PI3K activity will inhibit proliferation and induce apoptosis in cancer cells (Yao et al., 2020). BRAF V600E is activated by KRAS and it will phosphorylate MEK. Next, MEK will phosphorylate ERK. ERK travels to the nucleus and becomes a transcription factor for genes involved in tumor cell proliferation (O'Leary et al., 2019). Inhibition of BRAF V600E activity will stop this pathway and the uncontrolled proliferation of cancer cells can be stopped. JAK-STAT pathway will be activated when JAK3 is activated by EGFR. Then JAK3 phosphorylates STAT3/5 and forms a dimer. This STAT3/5 dimer then goes to the nucleus and becomes a transcription factor for genes such as Survivin, Mcl-1. Bcl-2, and VEGF have essential roles in cell proliferation and survival (Bose et al., 2020). By inhibiting JAK3 activity by peptide 11, this pathway will be inhibited so that proliferation does not proceed and stimulate cells to apoptosis (Wang et al., 2012). The peptides from *A. lixula* have high potential to inhibit NSCLC growth because they can inhibit three pathways at once in NSCLC (Fig. 3). By blocking the three pathways simultaneously, it is hoped that it can effectively inhibit the growth of NSCLC.

CONCLUSIONS

The peptides from *A. lixula* coelomic fluid have potential as anti-NSCLC agents because they can inhibit the activity of the EGFR, PI3K, BRAF V600E, and JAK3 proteins through the ATP inhibi-

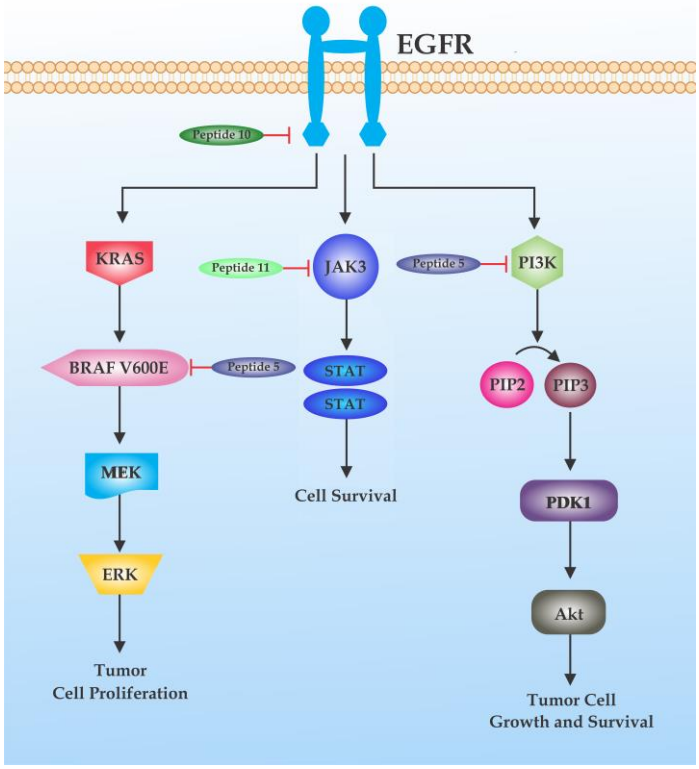

Figure 3. Peptides from *A. lixula* have the potential to inhibit three pathways in NSCLC.

tor mechanism. Inhibition of these proteins will inhibit three signaling pathways in NSCLC progression, namely the PI3K/Akt pathway, BRAF pathway, and JAK-STAT pathway.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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Contribution	Widyananda MH	Pratama SK	Samoedra RS	Sari FN	Kharisma VD	Ansori ANM	Antonius Y
Concepts or ideas	x	x	x	x	x	x	x
Design	x				x	x	x
Definition of intellectual content	x				x	x	x
Literature search	x	x	x	x			
Experimental studies	x	x	x	x	x	x	x
Data acquisition	x	x	x		x	x	x
Data analysis	x	x	x	x			x
Statistical analysis	x						
Manuscript preparation	x				x	x	
Manuscript editing	x				x	x	x
Manuscript review	x	x	x	x	x	x	x

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